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Original Article

Dentistry Section

Surface Morphology and Microbial Profile of Failed Peri-implantitis Affected and Clinically Healthy Dental Implants: A Comparative Cross-sectional Study

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ABSTRACT

Introduction: Dental implants are widely used for tooth replacement, but peri-implantitis remains a major cause of failure due to biofilm accumulation and destructive inflammation. Structural changes on implant surfaces may further enhance bacterial adhesion and compromise osseointegration. Despite the established role of surface characteristics and microbial factors in peri-implant disease, limited clinical evidence is available on their combined evaluation in failed implants compared with healthy implants, providing the rationale for the present study.

Aim: To compare the surface morphology and microbial load of failed peri-implantitis affected implants with clinically healthy implants using Scanning Electron Microscopy (SEM) and culture-based microbiological analysis.

Materials and Methods: This comparative cross-sectional study was conducted at the Department of Periodontology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India, from January 2025 to September 2025. A total of 20 participants were included, comprising 10 clinically healthy single-implant cases in Group 1 (control group) and 10 failed implants diagnosed with peri-implantitis in Group 2 (test group), selected based on defined clinical and radiographic inclusion criteria. Retrieved failed implants were subjected to SEM to evaluate surface alterations, with an unused implant of the same system examined as a structural reference for baseline surface morphology. Plaque samples collected from healthy and failed peri-implant sites were analysed using aerobic and anaerobic culture to quantify colony forming units (CFU/mL). Demographic parameters, including age and gender, were recorded for both groups. Intergroup comparisons were performed using the Independent t-test, while gender distribution was assessed using the Chi-square test, with the level of significance set at p-value <0.05.

Results: SEM evaluation using an unused implant as a qualitative structural reference demonstrated the baseline surface morphology of the implant system, including loss of micro-roughness, cracks, and adherent debris. Microbiological analysis comparing the clinical patient groups revealed significantly higher aerobic bacterial counts in the failed implant group (group 2: 6.61±1.02×106 CFU/mL) than in the clinically healthy implant group (group 1: $2.23\pm0.06\times10^6$ CFU/mL; p<0.001). Similarly, anaerobic counts were elevated in group 2 ($3.96\pm0.91\times10^6$ CFU/mL) compared with group 1 ($1.02\pm0.03\times10^6$ CFU/mL; p<0.001).

Conclusion: Failed peri-implantitis-affected implants exhibited substantial surface deterioration and significantly increased microbial load compared with healthy implants.

Keywords: Biofilm, Implantitis, Osseointegration, Surface topography

INTRODUCTION

Dental implants are a predictable and widely accepted modality for the replacement of missing teeth, providing excellent functional and aesthetic outcomes. Their long-term success depends primarily on the establishment and maintenance of osseointegration, defined as a direct structural and functional connection between bone and implant surfaces [1]. Although high survival rates have been consistently reported, implant success is influenced by multiple factors, including implant design, surface characteristics, surgical technique, systemic health status, oral hygiene practices, and loading conditions [2-5]. Despite advancements in implant materials and surface modifications aimed at enhancing osseointegration, biological complications continue to pose a significant challenge in implant dentistry.

Peri-implant diseases represent a major cause of implant failure and are broadly categorised into peri-implant mucositis and peri-implantitis. Peri-implantitis is characterised by inflammation of the peri-implant tissues accompanied by progressive marginal bone loss, ultimately jeopardising osseointegration [6]. Its aetiopathogenesis is multifactorial. Microbial colonisation plays a central role, with pathogenic species such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola strongly associated with disease progression [7]. Other contributory factors include poor oral hygiene, excess cement, smoking, systemic conditions such as diabetes, biomechanical overload, and a previous history of periodontitis [8,9]. Once established, peri-implantitis is difficult to manage because of the persistence of the biofilm and the complex topography of implant surfaces, which favours bacterial adherence [10].

The surface morphology of dental implants significantly influences both osseointegration and microbial attachment. Roughened and microtextured surfaces enhance bone anchorage but may also harbour greater bacterial loads when exposed to the oral environment, thereby contributing to peri-implant disease [11]. Evaluating surface changes in failed implants and correlating them with microbial profiles may provide valuable insights into the mechanisms underlying implant failure.

Although implant surface characteristics and peri-implant microbiology are individually well-established determinants of implant success and failure, their simultaneous evaluation on retrieved failed implants in direct comparison with clinically healthy implants remains inadequately explored. Most existing studies have assessed surface morphology or microbial profiles in isolation, often under experimental or in-vitro conditions. The present study integrates surface morphological assessment with culture-based microbial analysis of clinically retrieved implants, enabling a comparative understanding of surface alterations and microbial burden associated with implant failure. This combined approach provides clinically relevant insights into the interaction between implant surface characteristics and microbial colonisation, which may inform preventive strategies and guide improvements in implant surface design and maintenance protocols.

Accordingly, the present cross-sectional study aimed to comparatively evaluate the surface morphology and microbiological profile of failed peri-implantitis affected dental implants and clinically healthy dental implants using SEM and culture-based analysis. The objectives were to assess and compare surface morphological characteristics and microbial profiles between the two groups and to evaluate the association between surface alterations and microbial burden in failed implants.

The null hypothesis was stated that there would be no significant difference in surface morphology or microbial profile between failed and healthy implants, whereas the alternative hypothesis proposed that significant differences would exist between the two groups.

MATERIALS AND METHODS

This comparative cross-sectional study was conducted at the Department of Periodontology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India, from January 2025 to September 2025. Ethical approval was obtained from the Institutional Review Board (SRB/SDC/PERIO-2403/25/27), and written informed consent was secured from all participants before their inclusion in the study.

Sample size calculation: The sample size was determined using G*Power software (version 3.1.9.4) to provide 80% power to detect a significant difference in microbial load between healthy implants and peri-implantitis-affected implants, with a 95% confidence interval (α=0.05). The calculation was based on the Mean±Standard Deviation (SD) of microbial counts reported by Acharya A et al., where bacterial counts expressed as CFU/mL were $6.5\times10^5\pm0.5\times10^5$ and $1.25\times10^6\pm0.6\times10^6$ [12]. These values were used to estimate the expected effect size for intergroup comparison. Based on these parameters, a minimum sample size of 10 subjects per group was required. Accordingly, a total of 20 participants were included in the present cross-sectional study.

Inclusion criteria: Adults aged 30-50 years with single, bone-level, platform-switched dental implants restored with cement-retained porcelain-fused-to-metal prostheses after a minimum healing period of three months were included. All implants belonging to the same implant system (Dio®, Busan, Korea) were included. Clinical and radiographic assessments were performed for inclusion.

Exclusion criteria: Subjects with smoking habits, long-term antibiotic use, systemic illnesses, metabolic bone disorders, or a previous history of periodontitis were excluded.

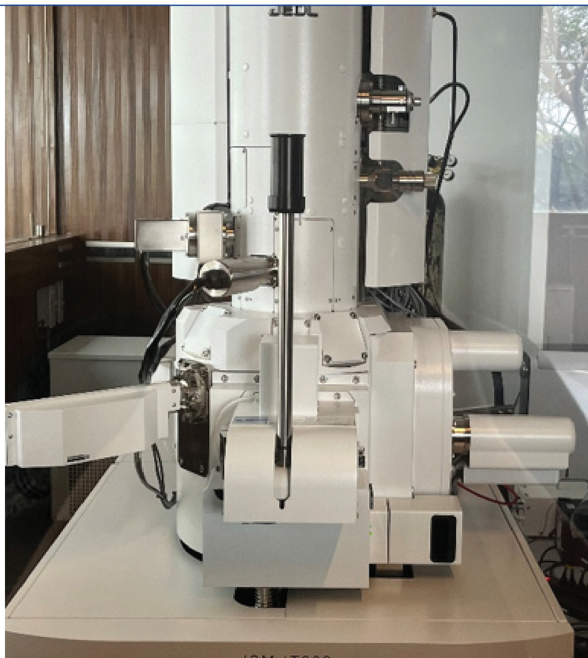
Study Procedure

A total of 20 participants were enrolled and allocated into two groups based on peri-implant tissue status according to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [13]:

- **Group 1 (Control; n=10):** Participants with clinically healthy implants exhibiting no bleeding on probing, probing depth ≤6 mm, absence of implant mobility, and no radiographic bone loss beyond physiological remodelling.
- **Group 2 (Test; n=10):** Participants with failed implants affected by peri-implantitis, exhibiting bleeding and/or suppuration on probing, probing depth ≥6 mm, implant mobility, and radiographic evidence of bone loss.

Preparation of implant specimens: Failed implants were retrieved atraumatically and immediately transferred to sterile containers to avoid contamination. Cleaning procedures were standardised to preserve the inorganic surface layer for subsequent analysis. The implants were immersed in 3% sodium hypochlorite for 10 minutes to remove organic debris, followed by immersion in pure acetone for 30 minutes. Ultrasonic agitation in a heated water bath was employed to enhance the cleaning efficiency. After each step, the implants were rinsed thoroughly with ethyl alcohol to minimise cross-contamination and prepare the samples for surface characterisation.

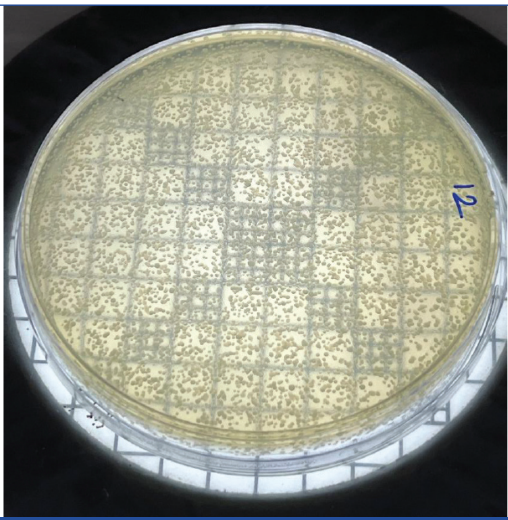
Surface topography analysis: Surface topography was examined using a JEOL Scanning Electron Microscope (JEOL JSM-IT800; JEOL USA, Peabody, MA) equipped with a Gemini column, offering a resolution of 1.5 nm [Table/Fig-1]. Before imaging, implants were sputter-coated with a thin gold layer (5-10 nm) under vacuum (approximately 0.05 mbar, 20 mA, 30-60 seconds) to improve conductivity and reduce charging. SEM evaluation was performed in high-vacuum mode at an accelerating voltage of 10 kV, with a working distance of 11.2 mm and a magnification of ×1000. Surface morphology was captured using an SE2 detector. An unused implant of the same brand was included as a structural reference for baseline surface morphology rather than as a clinical control for comparison with healthy implants.



[Table/Fig-1]: Scanning electron microscope.

Microbial analysis: Plaque samples were collected from the failed and healthy peri-implant sites using sterile paper points and immediately transported for culture. Sterile Brain Heart Infusion (BHI) broth was used as the transport and dilution medium. The samples were transferred into BHI broth, vortexed for homogenisation, and serially diluted in sterile saline. The diluted samples were then streaked onto BHI agar plates and incubated aerobically at 37°C for 24 hours in a 5% CO_2 atmosphere. After incubation, CFUs were quantified using a digital colony counter [Table/Fig-2] and expressed as CFU/mL after adjusting for dilution.

For anaerobic culture, plaque samples were placed in blood broth and stored at 4-8°C until processing. The samples were lawn-inoculated on blood agar plates and incubated in an anaerobic jar using a gas-pack system to achieve an oxygen-free environment [Table/Fig-3]. The plates were incubated at 37°C for seven days, after which CFUs were recorded and expressed as CFU/mL. All media were verified for sterility, and strict quality control measures were followed to ensure accuracy and reproducibility throughout the microbiological procedures.



[Table/Fig-2]: Digital colony counter for accurate enumeration of Colony Forming Units (CFU).

[Table/Fig-3]: Anaerobic jar with gas-pack system for oxygen-free incubation of blood agar plates for anaerobic culture.

STATISTICAL ANALYSIS

Data analysis was performed using Statistical Package for Social Sciences (SPSS) software version 23.0; (IBM Corp., Armonk, NY, USA). Normality of the data was assessed using the Shapiro-Wilk test, which confirmed a parametric distribution. Intergroup comparisons of age and microbial counts were carried out using an Independent t-test, while gender distribution between groups was evaluated using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

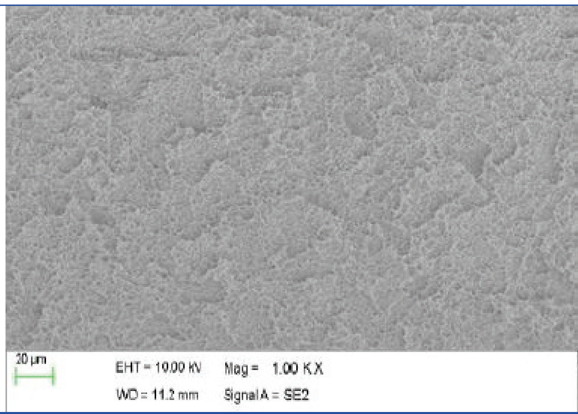
The mean age of participants in the healthy implant group was 38.6±4.2 years, while the failed implant group showed a mean age of 39.1±3.9 years, with no statistically significant difference between them (p>0.05). Gender distribution was also comparable between the groups, with six males and four females in the healthy implant group and five males and five females in the failed implant group, demonstrating no significant intergroup variation (p>0.05). These results indicate that both groups were demographically well matched [Table/Fig-4].

Parameters	Group 1 (n=10)	Group 2 (n=10)	Test value	p-value
Age (years), Mean±SD	38.6±4.2	39.1±3.9	t=0.28	>0.05†
Gender (Male/ Female)	6/4	5/5	χ^2=0.20	>0.05$^\gamma$

[Table/Fig-4]: Demographic characteristics of the study participants.
†Independent t-test; $^\gamma$Chi-square test

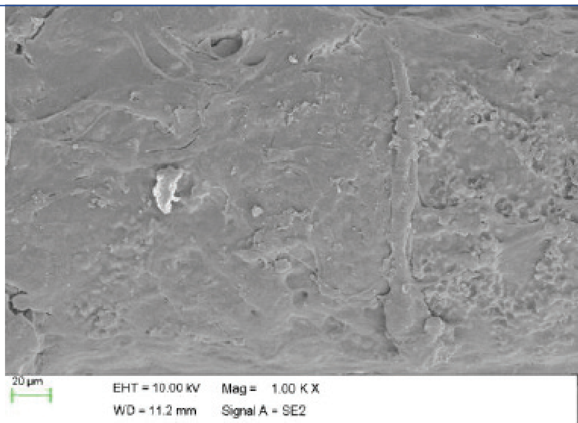
SEM Analysis

For qualitative baseline reference, the SEM micrograph of the unused implant shows a uniformly roughened surface with well-defined micro- and nano-scale irregularities. This textured topography reflects the original surface modification intended to promote osseointegration by enhancing cell attachment and increasing surface area. No debris, contaminants, or structural breakdown is evident, indicating an intact, pristine implant surface [Table/Fig-5].



[Table/Fig-5]: Surface topography of unused implant surface.

The SEM image of the failed retrieved implant reveals a markedly altered surface compared with the unused implant. The micro-roughness appears disrupted, with areas of smoothing and structural degradation. Irregular deposits, cracks, and surface debris are visible, suggesting long-term exposure to the oral environment and possible microbial colonisation. The loss of defined surface topography indicates biofilm accumulation, corrosion-related changes, and compromised surface integrity typically associated with peri-implantitis [Table/Fig-6].



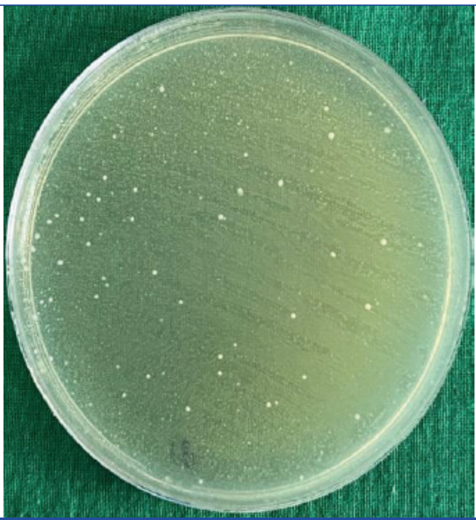
[Table/Fig-6]: Surface topography of failed implant surface.

Microbial Analysis

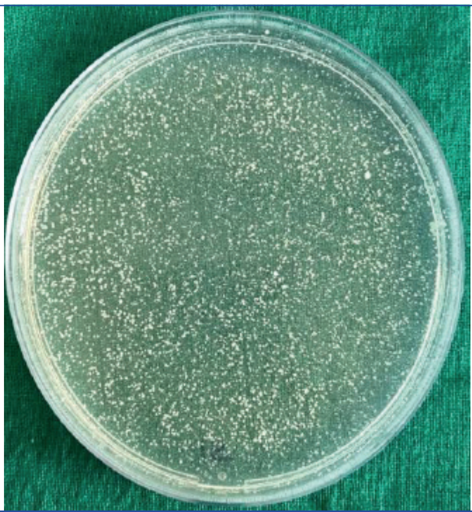
The microbiological analysis demonstrated a significant difference in bacterial load between the control and test sites. For aerobic cultures, the mean CFU/mL was 2.23±0.06×106 in the control group [Table/Fig-7] and 6.61±1.02×10^6 in the test group [Table/Fig-8], yielding a t-value of 13.56 and a p-value of <0.001, indicating a highly significant increase in aerobic bacterial load at diseased sites. Similarly, anaerobic cultures showed a mean CFU/mL of 1.02±0.03×10^6 in the control group [Table/Fig-9] compared with 3.96±0.91×10^6 in the test group [Table/Fig-10], with a corresponding t-value of 10.21 and a p-value of <0.001, confirming a statistically significant elevation of anaerobic bacterial load in diseased peri-implant sites [Table/Fig-11].

DISCUSSION

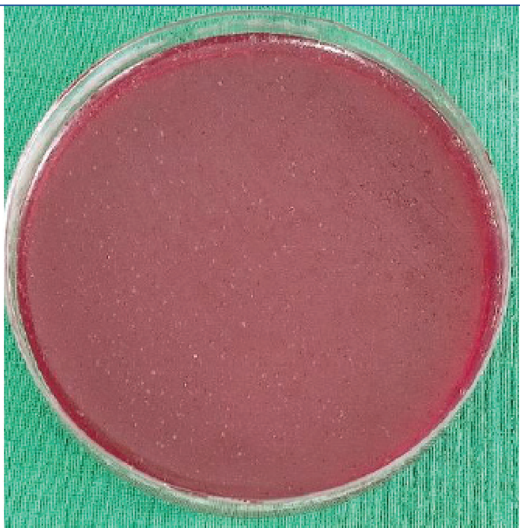
Peri-implant diseases continue to pose a significant challenge in implant dentistry, with peri-implantitis recognised as a major cause of late implant failure. Although the condition is multifactorial, emerging evidence highlights the combined role of surface alterations and



[Table/Fig-7]: Aerobic culture of healthy peri-implant site.



[Table/Fig-8]: Aerobic culture of failed peri-implant site.



[Table/Fig-9]: Anaerobic culture of healthy peri-implant site.

microbial colonisation in driving inflammatory breakdown around implants. Understanding how failed implants differ from healthy ones, both in terms of surface integrity and microbial ecology, is essential for improving diagnostic accuracy, predicting treatment outcomes, and formulating preventive strategies. Given these considerations, the present investigation focused on comparing the surface morphology and microbial profile of failed implants affected by peri-implantitis with those of clinically healthy implants. By employing SEM and culture-based microbial assessment, the study aimed to better elucidate structural changes on implant surfaces and the associated microbial burden that may contribute to implant failure.

[Table/Fig-10]: Anaerobic culture of failed peri-implant site.

Variables	Group 1 (Mean±SD)	Group 2 (Mean±SD)	t value	p-value†
Aerobic load (x10^6) (CFU/mL)	2.23±0.06	6.61±1.02	13.56	<0.001*
Anaerobic load (x10^6) (CFU/mL)	1.02±0.03	3.96±0.91	10.21	<0.001*

[Table/Fig-11]: Comparison of microbial counts between groups.
†Independent t-test;*Statistically significant

In the present study, failed implants demonstrated pronounced surface irregularities, including crater-like defects and disrupted surface continuity, whereas healthy implants exhibited smoother and more uniform morphologic characteristics. Microbiologically, both aerobic and anaerobic counts were significantly higher around failed implants than around healthy peri-implant sites, indicating a substantial microbial shift accompanying peri-implant disease progression.

The surface irregularities observed in the current study are consistent with the findings of Daood U et al., who reported that failed implants commonly exhibit crack-like defects and superficial craters that are likely generated during functional loading in the oral environment rather than during manufacturing [14]. Their description of numerous defects in the absence of instrument marks aligns with present study results and reinforces the notion that biological and mechanical stresses during service life may compromise implant surface integrity. Similarly, Shibli JA et al., and Secgin-Atar A et al., documented organic deposits, grooves, and ridges on failed implant surfaces, paralleling the morphological alterations observed in the present study failed implant samples [15,16].

The microbial analysis in the current study is also in agreement with existing literature. Kumar PS et al., demonstrated that peri-implant disease is associated with elevated levels of pathogenic species such as *Actinomyces, Campylobacter*, *Peptococcus*, and *S. mutans*, along with a reduction in commensal bacteria [17]. These microbial shifts were similarly reflected in our culture-based results, in which peri-implantitis sites harboured higher microbial loads than healthy sites. Findings from Tabanella G et al., further support this trend, showing an association between peri-implant bone loss and the presence of *T. forsythia, Campylobacter* species, and *P. micros* [18]. Additionally, pain and clinical signs of peri-implantitis were linked to the presence of *Fusobacterium* and *Eubacterium* species, reinforcing the pathogenic potential of these organisms.

Advanced sequencing-based investigations have likewise identified distinct microbial signatures in peri-implantitis. Kensara A et al., observed reduced alpha diversity and a higher abundance of Gram-positive species, particularly Enterococci, in implants affected by peri-implantitis compared with healthy controls [19]. This pattern is consistent with the dysbiosis observed in the present study, suggesting that both external and internal implant surfaces may serve as niches for pathogenic microorganisms. Furthermore, a systematic review by Sahrmann P et al., showed that no single species is exclusively responsible for peri-implantitis [20]; however, organisms such as *A.actinomycetemcomitans* and *P. intermedia* exhibit higher prevalence at diseased sites, echoing the polymicrobial nature identified in current study findings.

Overall, the present study reinforces the concept that peri-implantitis is associated with both surface degradation and microbial dysbiosis, highlighting the intertwined contribution of structural and biological factors to implant failure. Based on the statistically significant differences observed in both surface morphology and microbial load between failed and healthy implants, the null hypothesis was rejected. The findings confirm that peri-implantitis-affected implants exhibit distinct surface deterioration and significantly elevated bacterial burden compared with clinically healthy implants. A major strength of this study is the combined use of SEM and culture-based microbial analysis, which enabled a comprehensive evaluation of surface alterations and microbial burden associated with failed implants. The clinical implications of these findings indicate that peri-implantitis associated implant failure is characterised by a combination of surface degradation and increased microbial burden, emphasising the importance of early diagnosis, effective plaque control measures, and careful monitoring of peri-implant tissues. Altered implant surface characteristics may facilitate bacterial colonisation once exposed to the oral environment, underscoring the need for preventive maintenance protocols and timely intervention to limit disease progression. Future perspectives include longitudinal clinical studies to assess disease progression over time and investigations incorporating advanced molecular techniques to comprehensively characterise peri-implant microbial ecosystems and guide targeted preventive and therapeutic strategies.

Limitation(s)

Despite the strengths of the present study, certain limitations should be acknowledged. The cross-sectional design precludes assessment of temporal changes in implant surface characteristics and microbial profiles. The reliance on culture-based microbial analysis may not capture the complete diversity of peri-implant microbiota when compared with molecular sequencing methods. Additionally, potential confounding factors, such as variations in oral hygiene practices, occlusal loading patterns, and duration of implant function, could not be fully standardised or independently analysed and may have influenced the observed findings.

CONCLUSION(S)

Failed peri-implantitis affected implants showed distinct surface damage and significantly higher bacterial loads compared with healthy implants. These findings highlight the combined role of surface deterioration and microbial accumulation in implant failure and underscore the importance of early detection and preventive maintenance to improve implant longevity.

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