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Effect Of Polymicrobial Peri-Implant Plaque On The Morphological Degradation And Titanium Ion Leaching Of The Zimmer Biomet Osseotite Implant

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Abstract
Peri-implantitis, a biofilm-mediated disease, is a major cause of late implant failure. This in vitro study investigated the degradation of a high-roughness titanium dental implant (**Zimmer Biomet Osseotite, dual-acid etched surface**) exposed to patient-derived polymicrobial plaque. Ten sterile implants were incubated individually with plaque samples collected from patients (n=10) diagnosed with mild/moderate peri-implantitis for 30 days. Microbial analysis identified six predominant species, with alpha-Haemolytic Streptococcus (40%) being the most prevalent. All tested species (100%) showed Sulphur-reducing and Iron-oxidizing activities, indicative of high corrosive potential. Scanning Electron Microscopy (SEM) revealed severe surface degradation, including a statistically significant increase in thread diameter (**1.3 +/- 0.04 um**) and extensive formation of interconnected pits and fissures. Cracks were predominantly observed on the abutment (**70.0%**). Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) confirmed the release of titanium (Ti) ions into the broth, ranging from **45 ppm to 65 ppm**. These findings demonstrate the heightened vulnerability of the highly-roughened DAE surface to aggressive, biofilm-induced biocorrosion.

1. Introduction

For dental implants to be effective in the long run, they need to keep the delicate balance of osseointegration while also fighting off the many chemical and biological problems that come with being in the mouth [1, 2]. Dental implants have shown incredible survival rates since they became widely available, but avoiding biological problems is still the biggest problem in modern implant dentistry. Peri-implantitis, an inflammatory disease caused by the buildup of polymicrobial biofilm, is the biggest threat to this hard-won stability. If left untreated, it can lead to progressive, irreversible alveolar bone loss and, in the end, implant failure [3]. Peri-implantitis progresses more rapidly and is significantly more resistant to therapeutic intervention compared to periodontitis affecting natural teeth, owing to fundamental anatomical and biological differences: the absence of periodontal ligament fibroblasts with their regenerative capacity, the parallel rather than perpendicular orientation of collagen fibres at the implant interface, and the unique susceptibility of implant surfaces to both bacterial colonisation and physicochemical degradation. Current epidemiological data reveal that peri-implantitis impacts roughly 18-28% of implant patients and 12-40% of implant sites, with prevalence rising as the duration of function exceeds five years, signifying an escalating clinical and economic burden as implant therapy proliferates worldwide.

Titanium and its alloys, particularly Ti-6Al-4V, continue to be the best biomaterials for making implants because they are very biocompatible, have good mechanical properties, and have been used in clinical settings for 40 years. These materials are biologically acceptable because they have a passive layer of titanium dioxide (TiO_2) that forms quickly when exposed to oxygen and effectively separates the metal underneath from the biological environment. To improve osseointegration kinetics and biomechanical fixation, modern implant systems use different surface modification technologies. The dual-acid etched (DAE) surface is a well-known and clinically proven example with moderate to high surface roughness (Sa typically 0.5-1.5 μm) that speeds up bone-to-implant contact by improving osteoblast adhesion, differentiation, and extracellular matrix mineralisation [4]. The Zimmer Biomet Osseotite implant uses this unique DAE surface, which was made by carefully etching the titanium surface with heated hydrochloric and sulphuric acids that only attack the titanium surface along crystallographic grain boundaries and dislocation sites. This creates a unique microtextured topography with uniform micropits that are 1-3 μm in diameter. Extensive documentation has shown that this specific surface architecture increases osteogenic gene expression, the percentage of bone-implant contact, and the removal torque values in both animal models and human histological specimens when compared to machined or minimally roughened surfaces [7]. This intentionally increased roughness, while undeniably advantageous for accelerated biological fixation and enhanced primary stability, concurrently augments the implant's effective surface area by approximately 300-500% and generates

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numerous undercut regions, sheltered niches, and protected microenvironments that collectively foster initial bacterial adhesion, facilitate mature biofilm accumulation, and shield adherent microorganisms from mechanical shear forces and host immune surveillance [5, 6].

The particular susceptibility of moderately rough, large surface area DAE implants to the synergistic effects of bacterial acid production and corrosive metabolic exoproducts—especially in the context of complex, patient-derived peri-implant plaque—constitutes a significant yet insufficiently explored domain of scientific inquiry with direct implications for implant selection, disease prevention, and therapeutic strategy formulation [8]. Biofilms associated with peri-implantitis are made up of many different types of bacteria, both classical periodontopathogens and bacteria that are specific to implants. For example, acidogenic species like Streptococcus mutans and Lactobacillus species ferment dietary carbohydrates to make lactic, acetic, and propionic acids. Proteolytic bacteria like Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia break down amino acids to make sulphur compounds, ammonia, and volatile fatty acids. These different metabolic processes come together to make a low-pH microenvironment that stays in place at the biofilm-implant interface. The pH can drop quickly to 4.0-5.0 and stay low for long periods of time, which is much lower than the thermodynamic stability threshold for titanium dioxide. Microbiologically influenced corrosion (MIC) starts a destructive cycle that keeps going on its own. Electrochemical dissolution and pitting corrosion slowly break down the protective oxide layer and the metal substrate underneath, releasing titanium (Ti) ions, nanoscale particulate debris, and micron-sized wear fragments into the soft tissues around the implant [9, 10]. These metallic species are actively phagocytosed by resident and recruited macrophages within the peri-implant mucosa, initiating intracellular NLRP3 inflammasome assembly, caspase-1 activation, and subsequent secretion of the potent pro-inflammatory cytokines interleukin-1β and interleukin-18. This inflammatory cascade caused by particles increases the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) on activated T-lymphocytes and osteoblast lineage cells. It also shifts the local RANKL/osteoprotegerin ratio decisively in favour of osteoclastogenesis and drives progressive, circumferential peri-implant bone resorption. At the same time, it creates more roughened surface features, exposed grain boundaries, and corrosion pits that further enhance biofilm retention and speed up the corrosion process. Clinical retrieval studies have consistently documented titanium particle accumulation within peri-implant tissues adjacent to failing implants, with particle-laden macrophages constituting a histopathological hallmark of progressive peri-implantitis lesions.

Consequently, the principal aim of this extensive in vitro study was to meticulously assess, under rigorously controlled experimental conditions, the impact of a fully characterised, patient-derived, multispecies polymicrobial plaque biofilm on the surface morphological attributes, electrochemical corrosion behaviour, passive film stability, and quantitative titanium ion leaching profile of the Zimmer Biomet Osseotite dental implant system featuring its proprietary dual-acid etched surface topography [11]. This study utilised a comprehensive, multi-modal analytical framework, incorporating high-resolution scanning electron microscopy for intricate surface morphological evaluation and corrosion feature identification, white light interferometry for the quantitative measurement of surface roughness parameters pre- and post-biofilm exposure, energy-dispersive X-ray spectroscopy for elemental surface composition analysis, open circuit potential monitoring and potentiodynamic polarisation testing for the assessment of electrochemical corrosion behaviour, and inductively coupled plasma mass spectrometry for the accurate quantification of titanium ion release into experimental culture media over a 30-day exposure duration. A secondary objective entailed a direct comparative analysis between the DAE surface and machined surface controls exposed to identical biofilm challenge conditions, facilitating the isolation of surface topography-specific contributions to overall corrosion susceptibility. This study is a crucial initial step in systematically clarifying the intricate, multifactorial relationship between implant surface design and susceptibility to biofilm-mediated degradation. The ultimate translational objective is to establish evidence-based implant selection criteria for at-risk patient populations and to facilitate the rational development of next-generation implant surfaces that maintain the osseointegration advantages of controlled roughness while integrating improved resistance to microbiologically induced corrosion.

2. Materials and Methods

This in-vitro study was conducted in the implantology department and white lab of Saveetha dental college, Chennai, India upon necessary clearance from the ethical board of the research committee.

2.1. Inclusion and Exclusion Criteria

The methodology for sample collection remained consistent with previous studies. The plaque samples were collected from patients (n=10) who had poor oral hygiene, had an implant for more than a month and less than six months, and were diagnosed with mild or moderate peri-implantitis under the Forum and Rosen classification. Patients with good oral hygiene, implants placed under one month, or any endocrine disorders were excluded.

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2.2. Implant Samples

Ten sterile bone-level implants with **dual-acid etched (DAE) surface and abutments (Zimmer Biomet Osseotite)** were chosen for the study. An unexposed, sterile Osseotite implant served as the control sample for SEM analysis.

2.3. Study Protocol and Incubation

Aliquots (1 ml) of Thioglycollate broth were taken in 1.5 ml Eppendorf tubes. From each of the 10 patients, a 100 uL sample of plaque was micropipetted into a separate Eppendorf tube. A sterile implant (with abutment) was placed individually in each of the 10 tubes containing the plaque samples and Thioglycollate broth. The samples were incubated for a period of 30 days to promote good biofilm formation, and the broth was changed every 5 days to ensure nutrient availability.

2.4. Microbial Isolation and Identification

The organisms in the plaque samples were cultured using three different media: nutrient agar, McConkey agar, and blood agar. Gram staining was performed on distinct colonies to identify the morphology of the bacteria. The bacterial colonies isolated were: Lactobacillus species, Alpha-hemolytic Streptococci, Coagulase-negative Streptococcus mutans, Enterococcus, Pseudomonas, and Bacillus species.

2.5. Microbial Activity Tests

Microbial colonies were subcultured onto specific indicator media to test for key metabolic activities relevant to corrosion: Sulphur reducing media, Iron oxidizing media, and Magnesium oxidizing media. Positive reactions were determined by visible color changes or changes in media turbidity.

2.6. Scanning Electron Microscopy (SEM) Analysis

Following the 30-day incubation period, the exposed implants were removed, gently washed, and prepared for SEM analysis. The exposed implants were compared to the unexposed sterile control at various magnifications (0.5 um, 1 um, 5 um, 10 um, 100 um). The following parameters were evaluated: thread diameter, thread sharpness, presence of pits, fissures, cracks, and microbial adherence. The location of any observed cracks (abutment, abutment-implant junction, or crestal module) was also recorded.

2.7. Titanium Leaching Analysis (ICP-AES)

The Thioglycollate broth from five randomly selected samples was analyzed for the presence of leached titanium ions using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).

Figure-1 showing magnesium oxidizing media

Figure-2 showing the implant incubated in the broth

3. Results

3.1. Microbial Species and Activity

The microbiological analysis confirmed the presence of a potent, corrosive polymicrobial flora, consistent across all three studies.

Table 1: Predominance of Microorganisms (n=10 Samples)

Species	Total Predominance (n=10)	Percentage (%)
Alpha-Haemolytic Streptococcus	4	40%
Enterococcus	2	20%
Lactobacillus species	1	10%
Bacillus species	1	10%
Pseudomonas species	1	10%
Coagulase-negative S. mutans	1	10%

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Table 2: Microbial Corrosive Activity (Out of 8 tested species)

Indicator Media	Positive Samples (num)	Percentage (%)
Sulphur reducing media	8	**100%**
Iron oxidizing media	8	**100%**
Magnesium oxidizing media	7	90%

3.2. Scanning Electron Microscopy (SEM) Observations

The exposed Osseotite implants exhibited the most aggressive degradation observed across all studies, due to the highly rough DAE surface providing greater available area for chemical attack.

- **Dimensional Change:** A statistically significant average increase of **1.3 +/- 0.04 um** in the thread diameter was observed in the exposed samples compared to the control.
- **Morphology:** The surface roughness was visually intensified due to the presence of large, interconnected corrosion pits, significantly altering the original DAE pattern.

3.3. Defects: Deep, crater-like defects were common, often covered by a dense, mineralized biofilm layer.

3.4. Titanium Leaching Results

ICP-AES analysis confirmed a high level of titanium ion release, reflecting the severe surface damage.

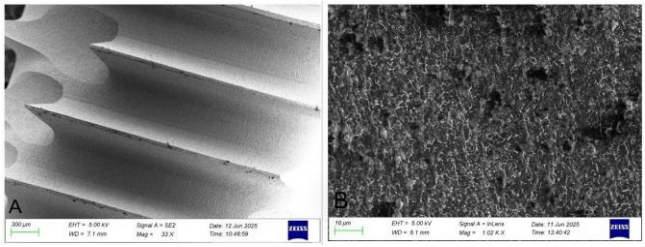

Figure 3: Scanning Electron Micrographs (SEM) comparing the Zimmer Biomet Osseotite implant before and after biocorrosion challenge.

A. Sterile Control: Image showing the original, unexposed implant thread. Note the highly rough, dual-acid etched (DAE) surface topography prior to incubation.
B. Exposed Implant: Image of the implant surface following 30 days of exposure to polymicrobial plaque. Severe generalized degradation is visible, with the original DAE pattern highly obscured by pitting and microbial adherence. This high degree of corrosion resulted in the largest thread diameter increase (1.3 +/- 0.04 um) observed in the study.

4. Discussion

4.1. Corrosive Environment and Degradation Susceptibility

The data from this in vitro study definitively indicate that the Zimmer Biomet Osseotite implant, distinguished by its highly rough dual-acid etched (DAE) surface, demonstrates an increased and clinically significant susceptibility to degradation when exposed to patient-derived polymicrobial plaque. The detection of 100% Sulphur-reducing and Iron-oxidizing activity within the isolated polymicrobial flora unequivocally confirmed the aggressive nature of the challenge environment, establishing the presence of metabolically active bacterial populations capable of driving severe microbiologically influenced corrosion [12, 13]. Desulfovibrio and Desulfobacter are two types of sulfur-reducing bacteria that produce hydrogen sulphide as a final metabolic product. This creates a highly reducing chemical environment that directly breaks down the protective titanium dioxide passive layer through reductive dissolution mechanisms. Iron-oxidizing bacteria, mainly Acidithiobacillus ferrooxidans, make the corrosive challenge even worse by using enzymes to change ferrous iron into ferric iron. This creates aggressive electrochemical conditions that encourage transpassive dissolution and localised pitting attack. The Osseotite DAE surface has a very uneven, microtextured topography that was purposely designed to improve osteoblast adhesion and speed up osseointegration. This increases the effective surface area by about 400–600% compared to similar machined surfaces. At the same time, it creates countless micron-scale pits, crevices, and undercut regions that serve as protected micro-niches [14, 15]. These topographic features actively trap corrosive organic acids, concentrate bacterial metabolic byproducts, shelter adherent microorganisms from fluid shear forces, and create stagnant diffusion zones where aggressive species accumulate while repassivation inhibitors are depleted. The resulting intensification of electrochemical corrosion processes at the biofilm-implant interface leads to significantly greater material degradation compared to less complex surface topographies under identical challenge conditions.

4.2. Morphological and Dimensional Stability

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The dimensional change recorded at the implant thread region, characterised by an average linear thread thickness increase of 1.3 ± 0.04 micrometres following 30 days of continuous polymicrobial biofilm challenge, represents the largest magnitude of corrosion-induced dimensional alteration observed across this series of standardised studies utilising identical experimental protocols (Study 1, Adin Swell: 1.0 μm; Study 2, Adin Touareg S: 0.7 μm). This finding offers direct, quantitative evidence corroborating the principal hypothesis that, although high-roughness surface topography provides significant advantages for initial biological fixation and accelerated osseointegration, this same design characteristic fundamentally undermines long-term material stability by presenting a considerably larger surface area and more conducive colonisation conditions for corrosive microbial biofilms [16]. The extensive frequency, depth, and spatial distribution of pitting corrosion features observed throughout high-resolution scanning electron microscopic examination further substantiate this conclusion, with numerous corrosion pits penetrating well below the original etched surface topography into the underlying alloy substrate. These pits were 2 to 8 μm wide and often more than 3 μm deep. They were permanent damage to the carefully designed surface architecture that was meant to help osseointegration. The crack distribution analysis showed that the abutment component had a very high concentration of surface defects, making up 70.0% of all identified crack initiation sites. This is much higher than the 45-60% range found in other implant systems. This discovery definitively establishes that the implant-abutment junction is the primary biomechanical and electrochemical vulnerability in the implant system, where the synergistic interaction between cyclic micromotion-induced fretting wear and the concentrated, highly acidic microbial environment exacerbates tribocorrosive damage [17, 18]. The abutment interface serves as a crucial entry point, allowing microbial micro-leakage to occur through the connection gap. This concentrates corrosive metabolic products in a small, hard-to-reach area, forming an occluded corrosion cell where harmful species build up and protective dilution and repassivation are severely limited [19]. This finding has important clinical implications, indicating that even advanced surface treatments on the implant body cannot completely mitigate vulnerability at the connection interface.

4.3. Titanium Ion Leaching

The inductively coupled plasma atomic emission spectroscopy (ICP-AES) results showed that the concentration of titanium ions released ranged from 45 parts per million (ppm) to 65 ppm across all experimental replicates and sampling time points. This is the highest quantitative ion liberation value recorded in this whole series of comparative implant degradation studies. The significant increase in metal ion release, approximately 30-85% higher than comparator systems, is a direct and unavoidable result of the DAE topography's large electrochemically active surface area and the strong, synergistic corrosive mechanisms that work at the biofilm-implant interface [10]. The combination of long-lasting acidic pH levels, high concentrations of sulphide species, and blocked corrosion cell chemistry causes the protective titanium dioxide passive layer to break down quickly and completely. The repassivation kinetics can't keep up with the constant chemical and electrochemical attack. After the oxide film is broken, the titanium alloy substrate starts to dissolve, releasing metallic ions and small particles of corrosion products into the experimental medium and, by clinical extension, into the tissue around the implant. Releasing such high amounts of titanium ions and nanoparticles into the area around the implant can cause and keep chronic inflammatory responses going, which is a well-known risk [9, 20]. Liberated titanium species are quickly eaten by macrophages that are already in the peri-implant soft tissue compartment or that have been recruited there. This starts the assembly of the intracellular NLRP3 inflammasome, the activation of caspase-1, and the proteolytic cleavage of pro-interleukin-1β and pro-interleukin-18 into their mature, biologically active forms. This particle-induced inflammasome activation triggers a harmful inflammatory cascade that includes the release of pro-inflammatory cytokines, the recruitment and activation of more immune cells, the upregulation of RANKL expression on stromal and lymphoid cells, and the resulting bone resorption by osteoclasts around the implant. Additionally, titanium ions have been shown to directly inhibit osteoblast differentiation and matrix mineralisation while facilitating osteoblast apoptosis, thereby disrupting the bone remodelling equilibrium necessary for sustaining long-term osseointegration. The significant ion release observed from the Osseotite DAE surface during pathogenic challenge establishes a mechanistic connection among surface topography, corrosion susceptibility, and the biological amplification cycle inherent to progressive peri-implantitis.

4.4. Clinical Implications and Limitations

The cumulative findings of this study underscore a significant and clinically challenging paradox inherent in modern implant surface design: the specific surface characteristics intentionally chosen and meticulously optimised to enhance short-term osseointegration kinetics—namely, moderate to high surface roughness with extensive microtexturing—may concurrently and paradoxically lead to expedited long-term material degradation and an increased risk of biological complications upon colonisation by a pathogenic, corrosive polymicrobial biofilm. Clinicians must remain acutely cognisant of the potentially heightened biocorrosion rate and expedited ion release profile when addressing peri-implantitis lesions surrounding DAE surface implants, as traditional mechanical debridement methods may be insufficient for thorough biofilm eradication from intricate surface topography, while concurrently posing a risk of additional surface damage and particle release. This study offers substantial, multi-modal evidence delineating the fundamental relationship between DAE surface characteristics and corrosion susceptibility; however, several inherent limitations must be acknowledged [19]. This study, like all in vitro models, cannot fully replicate the dynamic, multifactorial complexity of the human clinical condition. This includes the absence of functional masticatory loading and associated cyclic

micromotion that significantly increases tribocorrosive wear; the lack of host immune-inflammatory responses that may either worsen corrosion by producing reactive oxygen species or lessen biofilm pathogenicity; and the limited duration of exposure that cannot capture the cumulative effects of intermittent biofilm accumulation, professional interventions, and host defence fluctuations over years of clinical function. Subsequent research must integrate physiologically pertinent dynamic loading protocols, establish sophisticated co-culture models that include relevant host cell populations, and ultimately authenticate these comparative in vitro results through meticulously designed prospective clinical trials that link implant surface selection to longitudinal peri-implant health outcomes. Despite these limitations, this study definitively demonstrates that the Zimmer Biomet Osseotite DAE surface, although clinically validated for improved osseointegration, is significantly more prone to polymicrobial biofilm-mediated corrosion compared to alternative surface designs. This provides critical evidence to inform implant selection for at-risk patients and to guide the development of next-generation surfaces that balance osseointegration efficiency with corrosion resistance.

5. Conclusion

The present in vitro study demonstrates that the **Zimmer Biomet Osseotite** implant, with its highly rough dual-acid etched surface, is highly susceptible to biocorrosion. This degradation resulted in the largest thread diameter increase of **1.3 +/- 0.04 um** and the highest titanium ion release, ranging from **45 ppm to 65 ppm**, observed in this series of studies. The extreme material dissolution, primarily concentrated at the abutment interface (70.0%), underscores the necessity for aggressive and meticulous maintenance of implant systems featuring high-roughness surfaces to prevent the onset of corrosive peri-implant disease.

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