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Frequency of red complex microorganisms in dental implants with sandblasted acid etched and anodized surface by polymerase chain reaction

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ABSTRACT

Background Although surface topography and chemical composition of the dental implants greatly influences osseointegration, they also have an indirect impact on microbial adhesion. One important factor in the pathophysiology of peri-implantitis is thought to be bacterial adherence to the implant surface. This study aimed to compare the frequency of P. gingivalis, T. denticola and T. forsythia among SLA, SLActive and TiUnite implant surfaces using polymerase chain reaction.

Materials and methods Subjects with single healthy dental implant with cement retained prosthesis under function for at least one year were screened from Department of Implantology of Saveetha Dental College and Hospitals, Chennai. The eligible subjects were categorized based on the surface treatment into Group 1: SLA (n=25), Group 2: SLActive (n=25), Group 3: TiUnite (n=25). The presence of P. gingivalis, T. denticola and T. forsythia were evaluated in each of the three groups. Statistical analysis was performed using Fisher's exact test to determine the association between implant systems and bacterial frequency of red complex organisms.

Results The observed bacterial frequency pattern was TiUnite > SLA > SLActive. There was a significant association between implant systems and P. gingivalis ($p = 0.005$); with higher frequency of P. gingivalis was observed in TiUnite.

Conclusion Increased frequency of detection of red complex organisms around TiUnite dental implant as compared to SLA and SLActive implants. SLActive surface demonstrated lesser detection of red complex microorganisms, especially P.gingivalis as compared to SLA and TiUnite surfaces.

KEYWORDS: Acid-etched, Anodization, Dental implant, Microorganisms, Sandblasting

INTRODUCTION

In general, dental implants have provided long-term good results in the replacement of missing teeth. The first documented threaded titanium root-form implant was introduced in 1965 by Dr. Branemark(1). Ever since, implants have significantly evolved in terms of shape, size, and surface in order to optimize implant success and longevity. In spite of the great success rate of dental implants, some individuals experience initial implant failure from insufficient osseointegration and secondary implant failure from peri-implantitis(2).

Various surface modifications of implants are typically carried out to achieve and speed up osseointegration. These modifications are typically focused on modifying either the surface characteristics or the chemical composition. Acid treatments, sandblasting, or various oxidization mechanisms are used as the principal methods of surface modification for implants. Sandblasted-acid etched (SLA) implant surfaces are produced by sandblasting with coarse grit particles to change the implant's macrostructure and then etching the surface with an acid to induce micro-irregularitie(3). The implants are also hydroxylated, cleansed under nitrogen protection, and kept in an isotonic saline solution until they are used. This process creates a surface termed SLActive, an upgrade over SLA surface that has higher wettability(4). Another surface modification technique is anodization, which thickens the titanium dioxide layer by electrochemically altering the titanium implant surface(5).

Although the surface topography and chemical makeup of the implant can help with osseointegration, they also have a small but indirect impact on microbial adhesion. One important factor in the pathophysiology of peri-implantitis is thought to be bacterial adherence to the implant surface. The peri-implantitis onset and progression are driven by similar mechanisms as periodontitis(6). It is also believed that the causing microorganisms for peri-implantitis are comparable to periodontitis. It is well documented that red complex organisms, such as *Tan-*

neralla forsythia, Treponema denticola, and *Porphyromonas gingivalis*, become more prevalent during periodontal diseases(7,8).

Literature research reveals that most studies focus on the stability and survival of the implant. However, there are no studies assessing the frequency of pathogenic microorganisms among different implant surfaces. Therefore, this research was undertaken to compare the frequency of *T. forsythia*, *P. gingivalis* and *T. denticola* among SLA, SLActive and TiUnite implant surfaces using polymerase chain reaction.

MATERIALS AND METHODS

Study population

This study was conducted in compliance with the Helsinki Declaration of 1975, as updated in 2013, and was reviewed and approved by the Institutional Ethical Committee of Saveetha Dental College and Hospitals, Chennai. The study was conducted after each participant signed a consent form acknowledging their voluntary participation in the study.

Twenty-five subjects were identified in each of the following groups: Group 1: SLA (SLA®, Straumann, Basel, Switzerland), Group 2: SLActive (SLActive®, Straumann, Basel, Switzerland), Group 3: TiUnite (Nobel Biocare®, Gothenburg, Sweden). Eligible subjects were screened from the Department of Implantology of Saveetha Dental College and Hospitals, Chennai. Inclusion criteria: male and female participants aged between 25-60 years; at least 18 natural teeth excluding third molars; systemically healthy; periodontally healthy; single healthy dental implant with cement retained prosthesis in function for a minimum of one year. Periodontal and peri-implant health was determined based on the criteria delineated by the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases. Briefly, periodontal and peri-implant health was characterized by the absence of inflammatory changes with probing depths ≤3 mm.

Exclusion criteria considered were as follows: patients with systemic diseases like diabetes mellitus, cardiovascular diseases, hypertension, rheumatoid arthritis, pregnant and lactating women, patients under long-term medications including analgesics, antibiotics, immunosuppressants, bisphosphonates or steroids, smokers (current smokers and former smokers) and patients who had undergone oral prophylactic procedures within the last 3 months and patients with regular use of mouthwash.

Clinical examination

Probing pocket depth (PPD) is measured from the marginal gingiva to the base of the peri-implant sulcus, at six sites around the implant (mesial, mid and distal on both buccal and lingual surfaces) using a UNC-15 periodontal probe and the average was recorded.

Microbiological analysis

The supragingival plaque was removed using sterile curettes, and each chosen implant site was isolated with sterile cotton rolls. A sterile paper point was gently inserted into the sulcus depth and held there for 60 seconds. Until processing, the pooled subgingival samples were stored at -20°C.

The frequency distribution of *P. gingivalis*, *T. forsythia* and *T. denticola* was established using species specific primers [*P. gingivalis*, F: 5′-ACCTTACCCGGGATTGAAATG-3′, R: 5′-CAACCATGCAGCACCTACATAGAA-3′ (Amplicon size: 83 bp); *T. forsythia*, F: 5′-GGGTGAGTAACGCGTATGTA-ACCT-3′, R: 5′-GCCCATCCGCAACCAATAAA-3′ (Amplicon size: 127 bp); *T. denticola*, F: 5'-TAATACCGAATGTGCTCATT-TACAT-3', R: 5'-CTGCCATATCTCTATGTCATTG CTCTT-3'(Amplicon size: 860 bp)] under standard conditions. Using PureLink™ Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA), the genomic DNA was extracted in accordance with the manufacturer's recommendations. The following PCR procedures were carried out in a Bio-Rad T100 (BioRad, California, USA) thermocycler: one cycle at 94 ˚C for 5 min, 35 cycles at 94 ˚C for 30 s, 60 ˚C for 30 s, 72 ˚C for 1 min and a final cycle of 72 ˚C for 5 min. The annealing temperatures applied for each of the pathogens were: *P. gingivalis* 62 ˚C, *T. forsythia* 57 ˚C and *T. denticola* 57 ˚C. The amplicon obtained was electrophoresed on a 2% agarose gel and visualized under a UV transilluminator. The images were captured using the MegaCapt software.

Statistical Analysis

The data was analysed using the Statistical Package for Social Sciences (SPSS Software, Version 23.0; IBM Corp., Armonk, NY, USA). The results were evaluated using the Kolmogorov-Smirnov test and the Shapiro-Wilk test of normality. According to the data, the findings followed a parametric distribution. One-way ANOVA (analysis of variance) was used to compare mean age and PPD. Gender distribution was assessed using the Chi-square test.

	Group 1 (n=25)	Group 2 (n=25)	Group 3 (n=25)	p value
Age (years)	42.16±10.67	42.12±9.51	40.56±9.30	0.807a
Gender (M/F)	12/13	14/11	12/13	0.808b
PPD (mm)	3.78±0.57	3.64±0.40	3.96±0.64	0.130a

a Statistically insignificant (ANOVA)

b Statistically insignificant (Chi-square test)

TABLE 1 Demographic data of the study population

Similarly, the frequency of red complex organisms among three implant systems was assessed using the Chi-square test. Fisher's exact test was performed to confirm if there was any association between implant systems and bacterial frequency of red complex organisms. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

A total of 75 subjects Group 1: n=25 (SLA®), Group 2: n=25 (SLActive®), Group 3: n=25 (TiUnite®) participated in the present study. Table 1 summarizes the demographic and clinical characteristics of the study groups. There was statistical insignificance between the three study groups in relation to age (p = 0.807), gender (p = 0.808) and PPD (p = 0.130).

The frequency of *P. gingivalis*, *T. denticola* and *T. forsythia* was compared between the three groups (Table 2). The frequency of *P. gingivalis* was 16% in group 1, 0% in group 2 and 32% in group 3. The frequency of *T. denticola* was 16% in group 1, 8% in group 2 and 24% in group 3. The frequency of *T. denticola* was 8% in group 1, 8% in group 2 and 20% in group 3. The pattern of bacterial frequency observed was group 3 > group 1 > group 2. In group 3, there was a higher frequency of bacteria. The distribution of *P. gingivalis*, *T. denticola* and *T. forsythia* differed significantly (p = 0.000) amongst the three implant systems.

Final analysis was performed to determine the association between implant systems and the
frequency of each bacterial species (Table 3). There was a significant association between implant systems and *P. gingivalis* (p = 0.005); with higher frequency of *P. gingivalis* was observed in group 3. In addition, there was no significant association between implant systems and *T. denticola* (p = 0.363) and *T. forsythia* (p = 0.486).

Bacterial Frequency	Group 1	Group 2	Group 3	p value
P. gingivalis n (%)	4 (16%)	0	8 (32%)	0.000*
T. denticola n (%)	4 (16%)	2 (8%)	6 (24%)	0.000*
T. forsythia n (%)	2 (8%)	2 (8%)	5 (20%)	0.000*

** Statistically significant (Chi-square test)*

TABLE 2 Frequency of red complex organisms in SLA, SLActive and TiUnite implant surfaces

	Group		Absence	Presence	p value
P. gingivalis	1	Observed	21	4	.005*
		Expected	21.0	4.0	
	2	Observed	25	0	
		Expected	21.0	4.0	
	3	Observed	17	8	
		Expected	21.0	4.0	
T. denticola	1	Observed	21	4	.363
		Expected	21.0	4.0	
	2	Observed	23	2	
		Expected	21.0	4.0	
	3	Observed	19	6	
		Expected	21.0	4.0	
T. forsythia	1	Observed	23	2	.486
		Expected	22.0	3.0	
	2	Observed	23	2	
		Expected	22.0	3.0	
	3	Observed	20	5	
		Expected	22.0	3.0	

** Statistically significant (Fisher's exact test)*

TABLE 3 Association between implant systems and frequency of red complex organisms

DISCUSSION

The play of microorganisms in initiating the disease process around per-implant sulci has been under debate since the early 1990s. According to the findings, the surface topography and composition of the surface of the implant have a massive effect on microbial profile of implants, so it's plausible that the topographical disparities between different implant surfaces will affect the microbial profile(9,10). Despite decades of research, specific microbiological differences between the subgingival biofilms around different implant surfaces remain unclear. SLA, SLActive, and TiUnite are the three dental implants that are currently most frequently used in clinical settings. Each of the three dental implant surfaces has unique surface properties that encourage bone apposition. It is unclear which implant surface is least likely to harbor pathogenic microorganisms. This is the first study of its kind to compare the frequency of red complex microorganisms by polymerase chain reaction among SLA, SLActive, and TiUnite dental implants.

The present study revealed that there was an increased frequency of detection of red complex organisms around the TiUnite dental implant as compared to SLA and SLActive implants. Anodic oxidation is employed to prepare the TiUnite surface by generating a thick layer of TiO2. According to Albouy JP et al., implants with a TiUnite surface showed a greater disease progression than implants with a SLA surface. Additionally, implants with a TiUnite surface demonstrated a more significant progression of peri-implantitis and a less favorable treatment outcome than that seen around implants with a SLA surface(11). Furthermore, as compared to SLA implants, the findings of a prospective single-center clinical evaluation on 121 dental implants

with a TiUnite surface showed more significant tissue loss(12).
On the contrary, a recent meta-analysis revealed that the TiUnite surface had the best impact on osseointegration(13). Surface roughness is a crucial surface property for encouraging bone apposition, as is widely recognised. A parallel phenomenon is that biofilm formation is facilitated by increasing the surface roughness and surface free energy(14). All three of the systems examined in the current study feature relatively rough surfaces. Due to the different microbial colonisation, considerations other than surface roughness must be taken. The existence of grooves and pits in TiUnite's surface and its microdesign, which may shield bacteria from shear forces and facilitate bacterial adhesion, might account for the increased detection of red complex organisms(15).
The present study data showed that the SLActive surface demonstrated lesser detection of red complex microorganisms. Furthermore, there was a statistically significant difference in the frequency of detection of *P.gingivalis* around SLActive as compared to SLA and TiUnite surfaces. An upgradation to the SLA surface that provides more wettability is the SLActive surface. In order to prepare the SLActive surface, implants that have undergone SLA treatment are rinsed in a nitrogen environment and kept in NaCl solution as opposed to dry storage. Chemically altered implants have increased hydrophilicity, which inhibits hydrophobic contact and produces repulsion between the hydrophobic bacteria and the implant surface, inhibiting their adhesion and activity(16).
According to an in vitro study, periodontal pathogens like *P. gingivalis* have hydrophobic activity(17), which makes them less likely to adhere to hydrophilic surfaces(18). Additionally, it has been noted in a number of studies that the hydrophobicity of a surface may increase the propensity for microbes to adhere(19,20). Several studies have documented the greater extent of bacterial adhesion in hydrophobicity(21-23). On investigating the impact of surface chemistry on the adhesion of *S. aureus*, Tegoulia et al., found that the bacterial adhesion was higher on the hydrophobic surfaces(24). Furthermore, the degree of aerobic and anaerobic bacterial adherence was larger than on hydrophobic substrates(25).
In this study, dissimilarities in the frequency of red complex organisms were evident between the studied three implant surfaces, since higher frequencies were observed in the TiUnite implant surface. Our research also showed that pathogen occurrence near the SLActive surface appeared to be less common. Based on the observations, it was evident that there was a shift in microbial composition among dental implants with different surface treatments.
Collectively, these findings support the notion that the microbial profile is significantly influenced by the surface treatments applied to dental implants. The potential cause for microbial variability between various implant systems is structural variations and surface characteristics that may affect bacterial adherence. These microbial differences among implant surfaces, despite being negligible, might act as a potential threat to the rate of disease progression as peri-implantitis is expected to progress rapidly because of the absence of periodontal ligament fibers. In addition, this microbial disparity could affect the treatment strategies for peri-implantitis.
In summary, implants with different surface treatments may have a specific bacterial
microbiota. Quantification of these pathogenic microorganisms and the clinical significance of these findings should be analyzed.

CONCLUSION

Increased frequency of detection of red complex organisms around TiUnite dental implant as compared to SLA and SLActive implants. SLActive surface demonstrated lesser detection of red complex microorganisms, especially *P.gingivalis* as compared to SLA and TiUnite surfaces.

REFERENCES

1. Adell R. Tissue integrated prostheses in clinical dentistry. Int Dent Journal. 1985 Dec 1;35(4):259-65.
2. Chrcanovic BR, Albrektsson T, Wennerberg A. Reasons for failures of oral implants. J Oral Rehabil. 2014 Jun;41(6):443-76.
3. Barfeie A, Wilson J, Rees J. Implant surface characteristics and their effect on osseointegration. Br Dent J. 2015 Mar;218(5):E9.
4. Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, Hoffmann B, Lussi A, Steinemann SG. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res. 2004 Jul;83(7):529-33.
5. Sul YT, Johansson CB, Röser K, Albrektsson T. Qualitative and quantitative observations of bone tissue reactions to anodised implants. Biomaterials. 2002 Apr 1;23(8):1809-17.
6. Hultin M, Gustafsson A, Hallstro¨m H, Johansson LA, Ekfeldt A, Klinge B. Microbiological findings and host response in patients with peri-implantitis. Clin Implant Dent Relat Res. 2002;13(4):349-58.
7. Nowzari H, Botero JE, DeGiacomo M, Villacres MC, Rich SK. Microbiology and cytokine levels around healthy dental implants and teeth. Clin Implant Dent Relat Res. 2008 Sep;10(3):166-73.
8. Rajasekar A, Varghese SS. Microbiological profile in periodontitis and peri-implantitis: A systematic review. J Long-Term Eff Med Implants. 2022;32(4).
9. Grossner-Schreiber B, Teichmann J, Hannig M, Dörfer C, Wenderoth DF, Ott SJ. Modified implant surfaces show different biofilm compositions under in vivo conditions. Clin Oral Implants Res. 2009 Aug;20(8):817-26.
10. Berglundh T, Zitzmann NU, Donati M. Are peri-implantitis lesions different from periodontitis lesions? J Clin Periodontol. 2011 Mar;38:188-202.
11. Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Implant surface characteristics influence the outcome of treatment of peri-implantitis: an experimental study in dogs. J Clin Periodontol 2011; 38:58-64.
12. Ostman PO, Hellman M, Sennerby L. Ten years later. Results from a prospective single-centre clinical study on 121 oxidized (TiUnite) Branemark implants in 46 patients. Clin Implant Dent Relat Res. 2012; 14:852-860.
13. Hao CP, Cao NJ, Zhu YH, Wang W. The osseointegration and stability of dental implants with different surface treatments in animal models: a network meta-analysis. Sci Rep. 2021 Jul 5;11(1):1-2.
14. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res. 2006;17 (Suppl. 2):68-81.
15. Fickl S, Kebschull M, Calvo-Guirado JL, Hürzeler M, Zuhr O. Experimental peri-implantitis around different types of implants-A clinical and

radiographic study in dogs. Clin Implant Dent Relat Res. 2015 Oct;17:e661-9.
16. Yuan Y, Hays MP, Hardwidge PR, Kim J. Surface characteristics influencing bacterial adhesion to polymeric substrates. RSC advances. 2017;7(23):14254-61.
17. Naito Y, Tohda H, Okuda K, Takazoe I. Adherence and hydrophobicity of invasive and noninvasive strains of Porphyromonas gingivalis. Oral Microbiol Immunol. 1993 Aug;8(4):195-202.
18. Almaguer-Flores A, Olivares-Navarrete R, Wieland M, Ximénez-Fyvie LA, Schwartz Z, Boyan BD. Influence of topography and hydrophilicity on initial oral biofilm formation on microstructured titanium surfaces in vitro. Clin Oral Implants Res. 2012 Mar;23(3):301-7.
19. Zheng S, Bawazir M, Dhall A, Kim HE, He L, Heo J, Hwang G. Implication of surface properties, bacterial motility, and hydrodynamic conditions on bacterial surface sensing and their initial adhesion. Front Bioeng Biotechnol. 2021 Feb 12;9:643722.
20. Aykent F, Yondem I, Ozyesil AG, Gunal SK, Avunduk MC, Ozkan S. Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. J Prosthet Dent. 2010 Apr 1;103(4):221-7.
21. Parreira P, Magalhães A, Gonçalves IC, Gomes J, Vidal R, Reis CA, Leckband DE, Martins MC. Effect of surface chemistry on bacterial adhesion, viability, and morphology. J Biomed Mater Res Part A. 2011 Dec 1;99(3):344-53.
22. Cunliffe D, Smart CA, Alexander C, Vulfson EN. Bacterial adhesion at synthetic surfaces. Appl Environ Microbiol. 1999 Nov 1;65(11):4995-5002.
23. Absolom DR, Lamberti FV, Policova Z, Zingg W, van Oss CJ, Neumann AW. Surface thermodynamics of bacterial adhesion. Appl Environ Microbiol. 1983 Jul;46(1):90-7.
24. Tegoulia VA, Cooper SL. Staphylococcus aureus adhesion to self-assembled monolayers: effect of surface chemistry and fibrinogen presence. Colloids Surf B Biointerfaces. 2002 Apr 1;24(3-4):217-28.
25. Oh JK, Yegin Y, Yang F, Zhang M, Li J, Huang S, Verkhoturov SV, Schweikert EA, Perez-Lewis K, Scholar EA, Taylor TM. The influence of surface chemistry