Short Communication
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 18/06/2023;
Date Revised: 30/07/2023;
Date Published Online: 20/10/2023;

Authors' Affiliation:
1. Microbiology Department, College of Dentistry, University of Babylon, Babylon province - Iraq
2. College of Dentistry, University of Babylon, Babylon province - Iraq

***Corresponding Author:**
Hanan Selman Hessan
Email: dent.hanan.selman@uobabylon.edu.iq

How to Cite:
Hessan HS, Mohammed RAK (2023). In vitro evaluation of bacterial viability and adhesion of trio bacterial species on surface of titanium and zirconium dental implant abutment. Adv. Life Sci. 10S(1): 7-10.

Keywords:
S. mutans; *S. Sanguinis*; *Porphyromonas gingivalis*; Titanium abutments; Zirconium abutments

Open Access

In vitro evaluation of bacterial viability and adhesion of trio bacterial species on surface of titanium and zirconium dental implant abutment

Hanan Selman Hessan[1], Reyam Abdul Khuder Mohammed[2]

Abstract

Background: *Streptococcus mutans* and *Streptococcus sanguinis* are two species of bacteria belonging to the Streptococcus genus. Both S. mutans and S. sanguinis are part of the natural oral microbiota, but their roles and impacts on oral health differ. While S. mutans is associated with tooth decay, S. sanguinis helps maintain oral health by preventing the colonization of harmful bacteria.

Methods: Two species of *Streptococcus which are S. mutans* and *S. Sanguinis and the genus Porphyromonas gingivalis* were evaluated for their adherence and viability in vitro on Titanium, zirconium and dental implant surfaces, in addition to their individual screw. Two research groups were designed; 3 anvils of titanium included in group 1 and 3 anvils of zirconium included in group 2. The above groups were filtered into tubes containing cultures of bacteria, *S. mutans* and *S. sanguinis*, as well as *P. gingivalis* separately . The incubation time under anaerobic and anaerobic conditions was set at 37 °C for 24 hr. The adjustment in the number of colony-forming units of bacteria was tested for bacterial adherence (CFU). colorimetric test (Methylene blue test) was used for bacterial viability evaluation. For *S. mutans*, bacterial adhesion was greater in the titanium abutments (185.5 CFU/mL) and higher viability for *P. gingivalis* were published (71 %).

Results: The results showed that *S. mutans* recorded the best overall adherence (330 CFU/mL), while the best overall viability recorded with *S sanguinis*, was demonstrated in the zirconium abutment community (36.4 %). The greatest adhesion of *S. sanguinis* was demonstrated by the titanium screws (140.2 CFU/mL) . In contrast with the zirconium fixation screws, the greatest adhesion (144.3 CFU/mL) was observed for *S. mutans*. *S. mutans* recorded higher viability in both titanium and zirconium screws.

Conclusion: We may infer from this research that bacteria can bind to and thrive in both titanium and zirconium implants, as well as in fixation screws. *S mutans* demonstrated the strongest adherence to titanium and zirconium surfaces and fastening screws. In comparison, titanium abutments with *P. gingivalis* have greater bacterial viability than zirconium abutments with *S. sanguinis*. In both cases, as far as fixation screws are concerned, the feasibility of *S. mutans* was higher than the other bacteria. In titanium abutments greatest bacterial viability was recorded, while less bacterial adherence.

Introduction

A biofilm is a living bacterial colony made up of one or more species of bacteria that adhere to a solid surface. Pathogenic bacteria entering susceptible hosts is the first step in the pathogenesis of periodontal inflammation; other environmental variables also play a role in the disease's development [1]. Bacterial plaque accumulation is important for periodontal inflammation development and is essential stage for pathologies of other periodontal [2]. Unique bacteria have niche colonization sites in the oral cavity, according to Socransky [3], and their features are divided into main and secondary colonizers. Due to its significant connection with preimplantation diseases, *S. mutans* and *S. sanguinis* were listed as the major colonizers. [4], *P. gingivalis*, has also been included as a secondary colonizer[3]. The implant and the abutment form the root portion, which is a two-piece implant, from the conventional dental implant. Dental implants are considered the most effective management method for missing teeth replacement [5]. In the mouth microbiota if there is an imbalances between bacteria (pathogenic and nonpathogenic), resulting in rise in attachment to bacteria and therefore risk of periodontal infection chance increased, a great deal of which occurs. Mucositis of peri-implants and peri-implantitis are commonly observed [6]. Pathogenesis of peri-implant illness is caused by several factors including the systemic diseases, such as diabetes[7], the former history of tobacco [8] or periodontitis [9]. Conversely, there are many different factors that are involved with the peri-implant infection in which the root will need to be spiced-up. In order for the root to become infected, it needs to have bacteria in order to spark the infection. The discovery of adhesion of bacteria and different material abutments viability would help in an etiological disease understanding [10]. It was recorded by the prior meta-analysis peri-implantitis prevalence 9.83% and per implant mucositis prevalence of 29.48% [11]. The primary cause of bacterial resistance to conventional dental implants is the developing mucositis and periimplantitis in peri-implants. The dental abutment's surface characteristics will lead to adherence to the microorganisms [12, 13].

The current study aimed to determine the viability and adherence of different bacterial species to the zirconium and titanium surface abutments and, when exposed in vitro, to the fastening screws.

Methods

Our study included six cultures of bacterial with dissimilar strains of *S. sanguinis, S. mutans,* and *Porphyromonas gingivalis* were get from the central laboratory in the University of Babylon, using two distinct abutments of material, zirconium and titanium. The materials were sterilized beneath UV light for 15 minutes using cabin type II inside a laminar flow. Bacterial cultures of *S. mutans, S. sanguinis*, as well as *Porphyromonas gingivalis* separately cultured with an additional 10 percent sterile bovine blood in blood agar plates. According to the orders of the producer, the culture was applied. Plates having bacteria were incubated at 37 °C for 10 days for *Porphyromonas gingivalis* and for 3 days for *Streptococcus sanguinis and Streptococcus mutans* in the controlled anaerobic chamber supplied with Anaerocult reagent (Merch, USA) . On a 24-pit sterile Petri dish, the abutments of zirconium as well as titanium and their conforming fixing screws were installed and each pit with bacterial suspension (1000 µL) with the gage density of 0.5 McFarland was added. Then samples were incubated for 72 hours at 37 °C beneath a controlled anaerobic condition. Bacterial viability and adhesion were measured when the incubation period was completed. The adherence of bacteria was measured using CFU. A Serial dilutions were produced to get a little amount of bacteria in the specimen. Following that, a direct CFU microscopic count was done on all samples using the plate dissemination method [14, 15]. The Bacterial viability was measured via measuring the absorption values which was measured on the basis of the reduction of mitochondrial enzymes following colorimetric MTT tests by the ELISA reader (Bio-Rad)[16].

Results

The overall bacterial adherence (185.5 CFU/mL) in *S. mutans* subsequently values of the adherence (165.1 & 150.3) CFU/mL, respectively for *S. sanguinis* & *P. gingivalis* were revealed in our in vitro titanium abutment evaluation. With 71 %, *P. gingivalis* showed the highest bacterial viability value, while *Streptococcus mutans* and *Streptococcus sanguinis* showed 51 percent and 50 percent bacterial viability. A similar result was reported in a prior study comparing titanium alloy implants coated with titanium nitride *in vivo* versus implants that were not coated with titanium. After 24hr exposure to oral microbial, TiN-coated implants were found to have a minor quantity of surface enclosed by bacteria of oral cavity [17]. The highest bacterial adhesion of repairing screw was recorded for *Streptococcus sanguinis* (130.5 CFU/mL), *Streptococcus mutans* recorded the highest viability of bacterial (75.3%) with (330 CFU/mL) followed by *Streptococcus sanguinis* & *Porphyromonas gingivalis* with ethics of (131 and 223) CFU/mL, the *in vitro* evaluation of the zirconium abutment showed the highest bacterial adherence. *S. sanguinis* had 36.4% with respect to bacterial viability, followed by

Streptococcus mutans with 27.8 percent and *Porphyromonas gingivalis* with 24.2 percent.

Discussion

Previous studies have shown that zirconium oxide considered as low potential material for colonization & compared to titanium is lower adherence to bacteria is established with respect to zirconium abutments. Our results were disagreed with the previous studies, where less bacterial adherence recorded to titanium abutments than zirconium, while at the same time record greater viability of bacterial [18, 19]. A similar adhesion to *Streptococcus mutans* (144.3 CFU/mL), *Streptococcus sanguinis* (140.2 CFU/mL), & *Porphyromonas gingivalis* (102.1 CFU/mL) was shown in our fixation screw performance. There are some comparisons with *Streptococcus mutans* & *Streptococcus sanguinis* with ethics of (59.2 and 56.5) percent respectively in bacterial viability values. With 48.9 percent, lowest viability of bacteria was recorded for *Porphyromonas gingivalis*. Very few studies recognize, in the case of a fixation screw, the *in vitro* bacterial adhesion and viability of zirconia and titanium fastening screw happen when it is important to screw adjustment. Bacterial leakage between the abutment and the fastening screw occurs when the screw needs to be adjusted, according to Dibart *et al.* [20] and Nascimento *et al.* [21], But, it is suggested that further studies confirm whether the incidence of per implant disease can be increased by constantly changing the fixation screw; in this condition, it is essential to reduce the occurrence of bacteria in relative to the implant- abutment relationship. The physical parameters were the major disadvantages of this study, neither for pillars nor for screws, were not tested for zirconium and titanium surfaces. Moreover, since it was done in vitro, our results cannot be inferred to what could occur in the living tissues. As we could perform statistical tests with a larger sample number, sample size was also a constraint. Conversely, we are attentive on carrying out further research, taking into account different data, and *in vivo* experiments carried out in the future.

Author Contributions

The authors equally contributed in this study.

Competing Interests

The authors declare that they have no conflicts of interest.

References

1. Llambés F, Arias-Herrera S, Caffesse R. Relationship between diabetes and periodontal infection. World journal of diabetes, (2015); 6(7): 927.
2. Thurnheer T, Paqué PN. Biofilm models to study the etiology and pathogenesis of oral diseases. Oral Biofilms, (2021); 2930-37.
3. Nyvad B, Takahashi N. Integrated hypothesis of dental caries and periodontal diseases. Journal of oral microbiology, (2020); 12(1): 1710953.
4. Nagashima H, Shinoda M, Honda K, Kamio N, Watanabe M, et al. CXCR4 signaling in macrophages contributes to periodontal mechanical hypersensitivity in Porphyromonas gingivalis-induced periodontitis in mice. Molecular Pain, (2017); 131744806916689269.
5. Sun Y, Yang Y, Jiang W, Bai H, Liu H, et al. In Vivo Antibacterial Efficacy of Nanopatterns on Titanium Implant Surface: A Systematic Review of the Literature. Antibiotics, (2021); 10(12): 1524.
6. Zhou N, Huang H, Liu H, Li Q, Yang G, et al. Microbiota analysis of peri-implant mucositis in patients with periodontitis history. Clinical Oral Investigations, (2022); 26(10): 6223-6233.
7. Genco RJ, Graziani F, Hasturk H. Effects of periodontal disease on glycemic control, complications, and incidence of diabetes mellitus. Periodontology, (2020); 83(1): 59-65.
8. Sgolastra F, Petrucci A, Severino M, Gatto R, Monaco A. Periodontitis, implant loss and peri-implantitis. A meta-analysis. Clinical oral implants research, (2015); 26(4): e8-e16.
9. Ardelean LC, Rusu LC. Oral Health Care-An Important Issue of the Modern Society. (2022).
10. Deppe H, Ahrens M, Behr AV, Marr C, Sculean A, et al. Thermal effect of a 445 nm diode laser on five dental implant systems: An in vitro study. Scientific reports, (2021); 11(1): 20174.
11. Lee C-T, Huang Y-W, Zhu L, Weltman R. Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. Journal of dentistry, (2017); 621-12.
12. Han A, Tsoi JK, Rodrigues FP, Leprince JG, Palin WM. Bacterial adhesion mechanisms on dental implant surfaces and the influencing factors. International Journal of Adhesion and Adhesives, (2016); 6958-71.
13. Do Nascimento C, Pita MS, de Souza Santos E, Monesi N, Pedrazzi V, et al. Microbiome of titanium and zirconia dental implants abutments. Dental Materials, (2016); 32(1): 93-101.
14. Alvarado Pinedo MF (2022) Reconfiguración del diseño curricular del curso de Anatomía e Histología Básica para una nueva carrera de grado: desafíos de la enseñanza para un perfil profesional en construcción: Universidad Nacional de La Plata.
15. Caro-Hernández PA, Tobar JA. Análisis microbiológico de superficies en contacto con alimentos. Entramado, (2020); 16(1): 240-249.
16. Nga N, Ngoc T, Trinh N, Thuoc T, Thao D. Optimization and application of MTT assay in determining density of suspension cells. Analytical biochemistry, (2020); 610113937.
17. Zhurakivska K, Ciacci N, Troiano G, Caponio VCA, Scrascia R, et al. Nitride-Coated and Anodic-Oxidized Titanium Promote a Higher Fibroblast and Reduced Streptococcus gordonii Proliferation Compared to the Uncoated Titanium. Prosthesis, (2020); 2(4): 333-339.
18. Oda Y, Miura T, Mori G, Sasaki H, Ito T, et al. Adhesion of streptococci to titanium and zirconia. Plos one, (2020); 15(6): e0234524.
19. Alqutaibi AY, Alnazzawi AA, Algabri R, Aboalrejal AN, AbdElaziz MH. Clinical performance of single implant-supported ceramic and metal-ceramic crowns: A systematic review and meta-analysis of randomized clinical trials. The Journal of Prosthetic Dentistry, (2021); 126(3): 369-376.
20. Júnior RdAV, Aranha LC, Elias CN, Martinez EF. In vitro analysis of prosthetic abutment and angulable frictional

implant interface adaptation: Mechanical and microbiological study. Journal of Biomechanics, (2021); 128110733.
21. Vinhas AS, Aroso C, Salazar F, López-Jarana P, Ríos-Santos JV, et al. Review of the mechanical behavior of different implant–abutment connections. International Journal of Environmental Research and Public Health, (2020); 17(22): 8685.