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This article has been updated with language corrections.

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In vitro evaluation of bacterial viability and adhesion of three bacterial species on surface of titanium and zirconium dental implant abutments

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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 *Porphyromonas gingivalis* were evaluated for their adherence and viability in vitro on titanium and zirconium abutment surfaces, and their corresponding screws. Two research groups were designed: 3 abutments of titanium included in group 1 and 3 abutments of zirconium included in group 2. The above groups were placed into tubes containing cultures of bacteria, *S. mutans* and *S. sanguinis*, as well as *P. gingivalis* separately. The incubation time under anaerobic conditions was set at 37 °C for 72 h. The adjustment in the number of colony-forming units of bacteria was tested for bacterial adherence (CFU). colorimetric MTT assay was used, and absorbance was read using an ELISA reader 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* was observed (71%).

Results: The results showed that *S. mutans* showed the highest adherence on titanium abutments (185.5 CFU/mL), while the highest viability among zirconia abutments was recorded with S. sanguinis (36.4%). The greatest adhesion of *S. sanguinis* was demonstrated by the titanium screws (130.5 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 fixation screws. In comparison, titanium abutments with *P. gingivalis* showed higher bacterial viability (71%) than zirconia abutments with *S. sanguinis* (36.4%). In both cases, as far as fixation screws are concerned, the viability of *S. mutans* was higher than the other bacteria. In titanium abutments, greatest bacterial viability was recorded, while bacterial adherence was lower.

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 other periodontal pathologies [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 peri-implant 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 abutments 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 imbalance between bacteria (pathogenic and nonpathogenic), resulting in rise in attachment to bacteria and therefore risk of periodontal infection 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 adjusted. For the root to become infected, it needs to have bacteria 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 complications with conventional dental implants is the development of peri-implant mucositis and peri-implantitis, which promote bacterial colonization. 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 zirconium and titanium abutment surfaces and, under in vitro conditions, to fixation 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 under UV light for 15 minutes using Class II biosafety cabinet (laminar flow hood). 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 (Merck, USA). On a 24-well sterile plate/well, 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 adjusted to 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. Serial dilutions were produced to get a little number of bacteria in the specimen. CFU count was performed on all samples using the spread-plate method [14, 15]. Bacterial viability was measured via measuring the absorption values which were measured based on 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 and 150.3) CFU/mL, respectively for *S. sanguinis* and *P. gingivalis* was revealed in our in vitro titanium abutments evaluation. With 71 %, *P. gingivalis* showed the highest bacterial viability value, while *Streptococcus mutans* and *Streptococcus sanguinis* showed 51% and 50% 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 uncoated titanium. After 24 hr 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 fixation screw was recorded for *Streptococcus sanguinis* (130.5 CFU/mL), *Streptococcus mutans* recorded the highest bacterial viability (75.3%), with 144.3 CFU/mL followed by *Streptococcus sanguinis* and Porphyromonas *gingivalis* with ethics of 131 and 223 CFU/mL, in vitro evaluation showed that the highest bacterial adhesion was observed for S. mutans on titanium abutments (185.5 CFU/mL), whereas on zirconia abutments the highest viability was observed for S. sanguinis (36.4%). *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 zirconia is considered to have a lower potential for colonization than titanium, and lower bacterial adherence has been reported for zirconia abutments. Our results disagreed with the previous studies, where less bacterial adherence recorded to titanium abutments than zirconium, while at the same time recorded greater bacterial viability [18, 19]. A similar adhesion to *Streptococcus mutans* (144.3 CFU/mL), *Streptococcus sanguinis* (130.5 CFU/mL), and *Porphyromonas gingivalis* (102.1 CFU/mL) was shown in our fixation screw performance. There are some comparisons with *Streptococcus mutans* and *Streptococcus sanguinis* with values of 59.2% and 56.5% respectively in bacterial viability values. For titanium abutments, *P. gingivalis* showed the highest viability (71%), while S. mutans and *S. sanguinis* showed 51% and 50%, respectively. For fixation screws, S. mutans demonstrated the highest viability (75.3%), followed by *S. sanguinis* (131 CFU/mL; 56.5%) and *P. gingivalis* (223 CFU/mL; 48.9%). 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 pillars nor screws were 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 not perform statistical tests due to the small sample size, sample size was 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.

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