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Occurrence of Peri-Implant Microflora in Single vs. Two Piece Implants

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ABSTRACT

Objectives: Orthodontic mini-implants and dental implants become unstable in the event of peri-implant inflammation. The analysis of microbial colonisation in these implants would enhance the prolonged success rate of the implant approach. Thus, the present study aimed to determine the microbial colonisation in both single and two-piece implants on healthy individuals in order to elucidate the aetiology of infections following implant surgeries.

Materials and Methods: In all, five clinical samples were collected from mini-implants from patients undergoing orthodontic treatment and at least from two dental implants. The samples were collected using three 35# paper points. The colonies were identified and counted microscopically, and the number of viable microorganisms was calculated with respect to the number of colonies.

Results: *Streptococcus spp.*, *Lactobacillus casei*, *Candida spp.* and *Staphylococcus aureus* colonisations were identified based on the cell growth methods.

Conclusion: Microorganisms had accumulated around the dental implant and mini-implant surfaces before the healing post-abutment placement. Thus, further studies are essential on a variety of organisms to understand the mechanism underlying biofilm formation.

KEY WORDS

biofilm, bacterial adhesion, dental implant microbiology, mini-implants, culture media, peri-implantitis

INTRODUCTION

Approximately 80% of human microbial infections are related to medical implants¹⁾. The oral cavity is the primary source of bacteria in the human, and hence a hub for biofilm-related peri-implant diseases¹⁾. Bacterial infection has an effect on peri-implant bone loss²⁾. Thus, the present study aims to assess the microbiota in peri-implant tissues and mini-implants in order to elucidate the aetiology of infections following implant surgeries.

Nowadays, physicians and healthcare systems find the treatment of implant infection greatly challenging, as therapeutic interventions are frequently ineffective. Antibiotics are often insufficient, although some discovered compounds show efficiency. In orthodontics, mini-implants are commonly used as a temporary anchorage device, as they are favourable due to their arch wire engagement, compact size and low cost²⁾. However, the reduced success rate of mini-implants could be attributed to several factors, including oral hygiene and colonisation of pathogenic bacteria²⁾.

The oral cavity is inhabited by more than 700 different bacterial species³⁾. After placement, an implant's surface becomes coated with biofilm, and periodontal pathogens colonise the surface of the implants. After the miniscrew placement, the bacteria might spread to the peri-implant sulcus, which infects the soft and hard tissues and leads to conditions such as tissue inflammation, minor infection and peri-implantitis⁴⁾. These events are common in individuals with poor oral hygiene

post-implantation. Peri-implant soft tissue inflammation results in an approximately 30% increase in the failure rate of the implantation. Furthermore, infections associated with bone implant prostheses are caused by bacterial contamination. For instance, bacterial colonisation of the surface of the implant had been corroborated by the miniscrews extracted from orthodontic patients within three weeks of placement⁵⁾.

Peri-implantitis is a major biological complication responsible for the failure of dental implants⁵⁾. It has been defined as an infection of the mucosa surrounding the implants accompanied by loss of bone (detected clinically and radiographically), bleeding on probing, suppuration, epithelial infiltration and progressive mobility.

Strikingly, the increasing use of biomaterials and medical devices has led to an increased rate of the development of infection. Thus, bacterial biofilm contamination is a widespread problem in patients with dental implantation, and it is reportedly the most common cause of implant removal⁶⁾.

Biofilm formation is an example of dental plaque. In the oral cavity, the bacterial diversity colonises prosthetic devices, dental implants and mini-implants. Biofilm is composed of a population embedded in an extracellular polymer matrix with water channels. It protects and shelters the bacteria from the host defence mechanisms and detrimental substances in the surrounding environment. The growth of biofilm on implant occurs in two steps: (1) adhesion of early colonising bacteria, and (2) binding of secondary colonisers⁷⁾. Intriguingly, the surface components of the microbiota recognise the adhesive matrix molecules on the membrane of *Staphylococcus aureus*; this factor is pivotal for the

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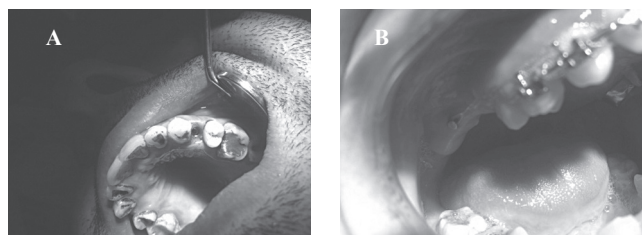


Figure 1. A: Patient treated with dental implant. B: Patient treated with mini-implant.

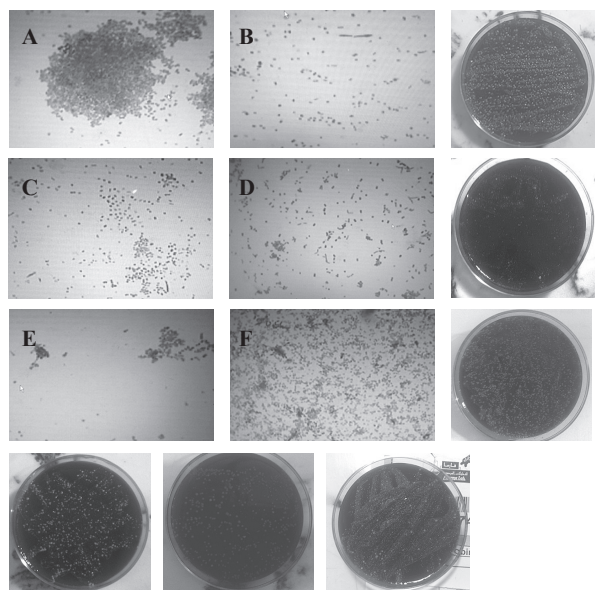


Figure 3. The biofilm inoculated onto Petri plates containing blood and MacConkey's, and Microscope used for identification of A. *Candida albicans*, B. *Lactobacillus*, and C. D. E. F. represented both *Streptococcus spp.* and *Staphylococcus aureus*.

recognition of and adhesion to surfaces. Viruses, fungi, protozoa and bacteria also interact with the medical device and are involved in the biomaterial contamination. Consequently, biofilm can protect the bacteria. Furthermore, *Streptococcus mutans* is one of the most significant species that has been identified in biofilm on oral implants. It elevates the inflammatory response and amplifies any bone defects. *Streptococcus gordonii* is a pioneer colonising species which adheres to both tooth and implant surfaces to initiate biofilm formation⁸. Recent studies have demonstrated that opportunistic fungal cells, such as *Candida albicans* and *Aspergillus*, are associated with non-responding antibacterial treatments⁹. Moreover, *S. aureus* was the most frequent microorganism isolated in human infection and on the surface of metallic devices¹⁰.

Presently, clinicians are addressing the issue of inflammation of the supporting tissues due to the colonisation of bacteria. The formation of biofilm on metallic devices is already a major concern in the biomedical field¹¹. Biofilm is a medical challenge because the antibiotics are often unable to diffuse inside the biofilm layer owing to the presence of the strains resistant to antibiotics¹¹. Thus, the present study aims to examine the microbial profile in the head, around the inner surface of the titanium implants before the placement of the prosthetic abutment and around the mini-implant.

MATERIALS AND METHODS

The ethics approval for the current study was granted by the Institutional Scientific Committee of the University of Anbar, Ramadi, Iraq under reference number 90 on 16/05/2018. Informed consent was

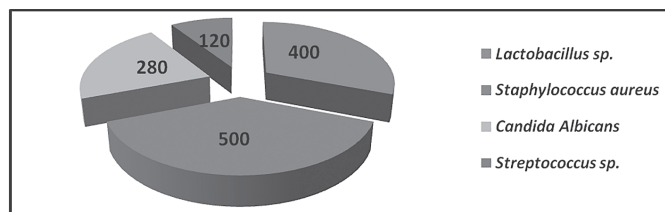


Figure 2. Detection and numbers of *lactobacillus spp.*, *Staphylococcus aureus*, *Candida albicans* and *Streptococcus spp.* from both healthy dental implants and mini-implants.

obtained from the participants. The patients were instructed to avoid food consumption and tooth brushing for one hour before the scheduled sampling session. Also, it was ensured that none of the individuals were suffering from any systemic diseases. All participants were examined clinically using a dental mirror and probe to detect the supragingival area around the dental implants and mini-implants. Subsequently, the supragingival plaque samples were collected using three #35 paper points and stored in 500 μ l of sterile saline. A total of two samples were taken from the dental implants (Germany, NuclOSS) and five samples of mini-implants were obtained from patients undergoing orthodontic treatment (self-drilling titanium mini-implants, 1.4 mm diameter x 6-8 mm long, 3M, Abson, Korea) from males aged 20-51 years. Then, the samples were transported to the laboratory. The samples were inoculated onto Petri plates containing blood and MacConkey's culture media, and the samples were then maintained at 37°C for 24 h in aerobic conditions. The identification and numbers of colonies was done by microscope. Numbers of microorganisms were calculated from the numbers of colony forming units.

RESULTS

All patients were examined clinically using a dental mirror and probe to detect the supragingival area around the dental implants and mini-implants. Subsequently, the supragingival plaque samples were collected as shown in Figure 1.

The pie-chart in Figure 2 represents the numbers of microorganisms based on the growth and morphology of the bacteria, which included: *lactobacillus spp.*, *Staphylococcus aureus*, *Candida albicans* and *Streptococcus spp.* These bacteria were detected on the mini-implant head and supragingival area as well on all the effective dental implants, as shown in Figure 2 and Figure 3.

DISCUSSION

Currently, dental implant treatment is a standard medical practice in dentistry. Furthermore, orthodontic appliances enhance oral hygiene, thereby altering the composition of bacterial plaque in patients. The failure in an early implant is commonly associated with specific bacteria, such as *Streptococci*, anaerobic Gram-positive cocci, and anaerobic Gram-negative bacilli as shown in Table 1. The formation of biofilm on the dental implant surface is already a major clinical issue. The initial biofilm development and ensuing colonisation include *Streptococcus spp.*, *Lactobacillus spp.*, and *Candida spp.*, which comprise the normal oral microbiota. *Staphylococcus* is isolated in the oral cavity and, hence, detected from the peri-implantitis. *Staphylococcus aureus* is also responsible for metallic biomaterial-related devices as well as medical infections¹². This microorganism also adheres to titanium surfaces¹². Furthermore, the bacterial colonisation into the implant-abutment interface in patients was examined using the DNA probe method¹³. The study reported that the implant-abutment was colonised by medium-to-high levels of eight presumed periodontal pathogens, which included *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

All individuals participating in the current study had healthy gingiva and had received post-surgical oral hygiene medication; none presented any clinical signs of gingivitis. A clinical examination of the individual was performed during every visit to evaluate the stability of

Table 1. Detection of different bacterial species in healthy peri-implant mucositis and peri-implantitis patients, and (successful and failure) orthodontic mini-implants, were analysed using different techniques.

Authors name	Findings	Samples	Techniques
(Shahabouee <i>et al.</i> , 2012)	Six anaerobic bacteria found in teeth and implant sulci were Gram-positive cocci, Gram-negative cocci, <i>Prevotella</i> , <i>Porphyromonas gingivalis</i> , <i>Bacteroid Fragilis</i> and <i>Fusobacterium</i>	Thirty-four partially edentulous patients with a total of 50 anterior maxillary single implants with cemented crowns and 34 similar teeth in the same jaw of the same patients were included	Dark field microscope
(Zheng <i>et al.</i> , 2015)	<i>Streptococcus</i> , <i>Leptotrichia</i> , <i>Capnocytophaga</i> , <i>Prevotella</i> , <i>Fusobacterium</i> , <i>Neisseria</i> and <i>Rothia</i> genera were dominant in 24 samples. <i>Porphyromonas gingivalis</i> , <i>Tannerella forsythia</i> and <i>Prevotella intermedia</i> were clustered in the peri-implant mucositis	Ten healthy peri-implant site individuals, 8 cases with peri-implant mucositis and 6 cases with peri-implantitis	Polymerase chain reaction
(Gürlek <i>et al.</i> , 2017)	<i>Actinomyces naeslundii</i> and <i>Streptococcus oralis</i> in healthy implants. <i>Prevotella intermedia</i> and <i>Treponema denticola</i> lowered in mucositis. <i>Treponema denticola</i> increased in peri-implantitis	Ninety-seven implants and teeth (58 implants [19 healthy, 20 with mucositis, 19 with peri-implantitis] and 39 natural teeth [19 healthy, 12 with gingivitis, 8 with periodontitis] in 15 systemically healthy patients.	Immunoassay and real-time polymerase chain reaction
(Lafaurie <i>et al.</i> , 2017)	<i>Porphyromonas gingivalis</i> and <i>Prevotella intermedius/nigrescens</i> , uncultivable asaccharolytic anaerobic Gram-positive rods, anaerobic Gram-negative rods and rarely enteric rods and <i>Staphylococcus aureus</i>	Twenty-one of the articles evaluated the microbiologic profile of peri-implantitis versus healthy implants or periodontitis	Culture technique
(Guarnieri <i>et al.</i> , 2016)	<i>Aggregatibacter actinomycetemcomitans</i> , <i>Porphyromonas gingivalis</i> , <i>Prevotella intermedia</i> , <i>Treponema denticola</i> and <i>Tannerella forsythensis</i>	Subgingival plaque samples were collected from 17 patients (11 periodontally healthy and 6 periodontally compromised)	Real-time polymerised chain reaction (RT-PCR)
(Neilands <i>et al.</i> , 2015)	The microbial composition was higher in both healthy and peri-implantitis, but <i>Porphyromonas/Prevotella</i> and anaerobic Gram-positive cocci were more dominant in peri-implantitis	Twenty-five healthy subjects and 25 subjects with peri-implantitis	Culture technique
(Montebugnoli <i>et al.</i> , 2015)	The presence of <i>Treponema denticola</i> , <i>Porphyromonas gingivalis</i> , <i>Prevotella intermedia</i> , <i>Tannerella forsythensis</i> and <i>Actinobacillus actinomycetemcomitans</i> do not differ from those observed in healthy cases after first-year loading	Subgingival samples were taken from 13 organ-transplanted patients and 13 healthy individuals who received 29 and 28 submerged dental implants	Polymerase chain reaction
(Salvi <i>et al.</i> , 2012)	No differences in the detection of putative periodontal pathogens between implant and tooth sites	Fifteen subjects with healthy or treated periodontal conditions and restored with dental implants	DNA-DNA hybridisation
(Mengel <i>et al.</i> , 2005)	<i>Actinobacillus actinomycetemcomitans</i> , <i>Porphyromonas gingivalis</i> , and <i>Prevotella intermedia</i> were detected at teeth and implants	Thirty-nine partially edentulous patients: 15 cases treated for generalised aggressive periodontitis, 12 cases treated for generalised chronic periodontitis and 12 healthy patients	Dark-field microscopy
(Giannopoulou <i>et al.</i> , 2003)	There was no significant change in microbiology and biochemical parameters	Sixty-one implants in 45 systemically healthy patients	Dark-field microscopy and immunofluorescence
(Shibli <i>et al.</i> , 2008)	Higher numbers of <i>Porphyromonas gingivalis</i> , <i>Treponema denticola</i> and <i>Tannerella forsythia</i> were identified in the peri-implantitis compared with healthy patients	Forty-four subjects: 22 with peri-implantitis and 22 healthy patients	Checkerboard DNA-DNA hybridisation
(Andrucioli <i>et al.</i> , 2018)	All 40 microbial species were detected in both successful and failed mini-implants	Fifteen successful and 10 failed mini-implants	Checkerboard DNA-DNA hybridisation
(Ferreira <i>et al.</i> , 2015)	Extensive bacterial colonisation on mini-implant heads and transmucosal profiles were observed in successful and failed mini-implants	Twelve patients undergoing orthodontic treatment: 7 successful and 5 failed mini-implants	Scanning electron microscopy
(Tortamano <i>et al.</i> , 2012)	<i>P. intermedia</i> , <i>A. actinomycetemcomitans</i> and <i>P. gingivalis</i> were detected in both groups	Thirty-one mini-implants: 16 mini-implants without mobility and 15 mini-implants with mobility.	Polymerase chain reaction.

(de Freitas <i>et al.</i> , 2012)	<i>Streptococcus spp.</i> , <i>Lactobacillus casei</i> and <i>Candida spp.</i> colonisations were detected but <i>Porphyromonas gingivalis</i> was not detected	Fifteen mini-implants	Cell-growth methods and Polymerase chain reaction
(Apel <i>et al.</i> , 2009)	Four <i>Actinomyces viscosus</i> and three <i>Campylobacter gracilis</i> were detected in successful and rarely found both species in failed mini-implants	Eight failed mini-implants and 4 successful mini-implants	Real-time quantitative polymerase chain reaction in combination with a microarray-based identification of 20 chosen species

the anchorage device, since the purpose of the present study was to identify the microorganisms surrounding the orthodontic mini-implants and dental implants under healthy conditions. After the insertion of the mini-implants in the attached gingiva, the microbial colonisation site is created. Rajesh *et al.*⁵⁾ reported that the implants in partially edentulous patients have a greater risk of peri-implantitis than in completely edentulous patients; this is because in partially edentulous patients, the natural teeth act as reservoirs for oral pathogens to colonise the implants in the same mouth¹⁴⁾.

Nevertheless, the miniscrews placed in the mandible increased the risk of failure fivefold compared to those placed in the maxilla¹⁵⁾. Hitherto, only one study has provided information on the risk factors for heightened failure rates of mini-implants. It may be noted here that only inflammation has been recognised as a factor that increases the risk of failure by 4.8 times¹⁵⁾. Thus, it can be deduced that, for successful implants, the surrounding inflammation should be prevented¹⁾.

Existing studies have used culture-based methods, 16S rRNA gene PCR or DNA-DNA hybridisation methods that offered negligible evidence on the total diversity of the peri-implantitis milieu. However, it has been possible to garner deep insights on the structure of the microbiota in the oral cavity of the healthy individuals, as well as in the case of diseases, by sequencing the 16S ribosomal genes. Furthermore, the major genera represented in healthy oral cavities includes: *Streptococcus*, *Veillonella*, *Granulicatella*, *Gamella*, *Actinomyces*, *Corynebacterium*, *Rothia*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Capnocytophaga*, *Nisseria*, *Haemophilis*, *Treponema*, *Lactobacterium*, *Eikenella*, *Leptotrichia*, *Peptostreptococcus*, *Staphylococcus*, *Eubacteria* and *Propionibacterium*¹⁶⁾.

A study on peri-implant bacterial communities employed 16S pyrosequencing and proposed a broad-spectrum microbial profile of healthy implants compared to that of the peri-implant sites¹⁷⁾. In addition, 16S rRNA gene clones demonstrated higher proportions of *Actinomyces*, *Gemella*, *Kingella* and *Rothia* and lower levels of *Campylobacter*, *Desulfobulbus*, *Dialister*, *Eubacterium*, *Filifactor*, *Mitsukella*, *Porphyromonas* and *Pseudoramibacter* in healthy implants¹⁸⁾. Consequently, some studies did not detect any difference in the microbial diversity between peri-implants and healthy sites¹⁹⁾, while other studies identified fewer species in healthy sites in comparison with peri-implantitis sites. Some studies stated that the colonisation of the mini-implant sulcus did not grow remarkably since the devices were composed of biocompatible titanium alloy, which could have prevented the adherence of microorganisms. Thus, the microbiota might be linked with the development of peri-implantitis in periodontal disease. Previous investigations detected that the peri-implant biofilm in healthy subjects did not differ from the subgingival biofilm in disease²⁰⁾. Lee *et al.*²¹⁾ observed the microbial changes in implants that had been in function longer and saw in those patients a history of periodontal infections and peri-implantitis.

Typically, bacteria were detected in periodontal patients, although the same bacteria were isolated from healthy individuals. In the present study, healthy individuals did not display any symptoms of periodontal inflammation and presented successful temporary anchorage devices. Thus, any anaerobic bacteria could not be identified. Taken together, these findings of the present study confirmed that after the mini-implants were exposed to the oral cavity, colonisation ensued in the initial 24 hours.

CONCLUSION

Microorganisms accumulated around the dental implants and

mini-implant surfaces before the healing post-abutment placement. Although we concluded the presence of *Candida albicans*, *Streptococcus spp.*, *Lactobacillus casei*, and *Staphylococcus aureus* in the oral environment, it can be deduced that the simple approach of the present study might have rendered bias in the identification of the microorganism. Thus, further studies are essential with respect to anaerobic cultures in order to identify multiple species. However, several clinical samples and investigations are imperative to elucidate the process of biofilm development.

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