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Original Research

Evaluation of changes in microbial flora in delayed and immediately placed dental implants: A comparative study

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ABSTRACT:
Background: Peri-implant mucositis or implantitis is considered to be associated with pathogenetic micro-organisms present in the sulcus of implant and bone interface. Although, the distinct variations in the microbial colonization that result in the failure of implants eventually need to be studied and analyzed. **Aim**: The objective of this study was to evaluate alterations in microbial flora associated with delayed and immediately placed implants and compare the differences. **Materials and methods**: Total numbers of 300 implants (n=150, delayed and n=150, immediately placed) were assessed for alterations in the microflora population in different stages i.e., baseline, S1, following the first surgery, follow-up, second-stage surgery, follow-up, abutment placement, and follow-up. The most frequent organisms were the Streptococcus species. Other organisms included F. nucleatum, P. intermedia, A. actinomycetemcomitans, P. intermedia. **Results**: No statistical difference was observed between microbial growths in both the implant placement methods.
Keywords: Delayed, immediate, implants, microflora.

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INTRODUCTION

Soft tissue that surrounds an implant is termed as ‘peri-implant mucosa’ whereas the soft tissue that surrounds teeth is called ‘gingiva’. Though there is a morphological similarity between these tissues, histological differences or variations are found between them. [1] Soft tissue surrounding an implant consists of keratinizing as well as non-keratinizing epithelium that has alikeness to periodontal tissues around any natural teeth. Unlike a natural tooth, no periodontal ligament exists between an implant and bone surrounding it. The implant to bone contact has no direct neural or vascular supply. Since dental implants have a direct connection with surrounding bone tissue, the masticatory forces that are applied over an implant cannot be counteracted. Additionally, peri-implant tissues completely lack mechano-receptors within the periodontal ligament region that are responsible for the sensation of touch. [2]

Pathological alterations or changes that occur within tissues surrounding an implant are collectively called 'peri-implant diseases’. When these inflammatory alterations are limited within soft tissues, they are named "peri-implant mucositis” while when they spread to the underlying alveolar bone under soft tissues, the term ‘peri-implantitis’ is used. [3]

“Peri-implantitis” is a plaque-induced disease and is a late-occurring complication that can affect osseointegration of implants that may lead to progressive loss of bone and destruction of soft tissues. It is an important factor that results in loss of implants and has been associated with a specific type of micro-organisms that may be termed as ‘periodontopathic’ bacteria. [4]

Peri-implant diseases following successful osseointegration of bone with implant surfaces are caused by an imbalance between bacterial activity and host response. The response to inflammation in peri-

implant diseases might be confined to mucosal tissues surrounding an implant such as in 'peri-implant mucositis' or else there might be progression to supporting bone tissue loss that can cause in 'peri-implantitis'. [5, 6]

Microbiological profiling of peri-implantitis has been demonstrated to exhibit major periodontopathic micro-organisms associated with bacteria belonging to the 'red complex' as the main component. These are Porphyromonas gingivalis, T. forsythia as well as Treponema denticola .[7]

Dental implants fail to undergo osseointegration in sites where the failure of endodontic treatment has occurred. This could be a result of microbial colonization by a variety of anaerobic as well as facultative bacterial organisms. Thus, if implant placement is done at a site wherein bacteria colonization is present, there might be a failure in the integration of the implant due to coronally directed colonization of bacterial species. If an implant-associated cortical bone is thinned or there is apical fenestration, the colonization may proceed through the thin or overlying mucosa thus, providing relief in inflammation-related pressure for creating any apically directed or retrograde peri-implantitis. Thus, Enterococcus faecalis is the main suspect responsible for failures of implants. After performing complete debridement, an implant can be placed immediately following extraction of a tooth with endodontic failure while the patient is being treated with any appropriate antibiotics. The surface of an implant might undergo colonization whenever there is the exposure of implant surface to bacteria. Thus, performing good quality of debridement is important. However, there might be the persistence of micro- organisms. [7]

Thus, complete profiling of oral microbiota that has been associated with peri-implant diseased tissues must be done for the understanding of various responses by host factors that are associated with genetics along with the environmental origin and must move towards developing various diagnostic, preventive as well as therapeutic reasons.

Both mucositis, as well as peri-implantitis, are common microbial biofilm-associated oral diseases that affect tissues surrounding a dental implant. These diseases have significant medical as well as socio-economic impacts. Peri-implantitis related microbiome has site-specificity when compared with the microbiota of healthy implant systems. Implant-associated mucositis is especially rich for 'Fusobacterium nucleated that acts as a mile-stone colonizer related to implants.

Thus, based upon these facts the study aimed to comparatively evaluate alterations in oral microbial flora in delayed and immediately placed implants.

MATERIALS AND METHODS

This study was conducted upon patients who visited the dental out-patient department of the institute with the chief complaint of prosthetic replacement of missing teeth with implants. Microbial analysis was performed at the Department of Microbiology. The total study sample size was comprised of 300 implants of which 150 cases had immediately loaded implants and 150 cases had delayed placement of implants. Institutional ethical clearance was obtained and written informed consent was obtained before starting the implant placement procedure. Materials, as well as culture media used, were previously sterilized. Scaling as well as polishing of dentulous regions was performed one day before collecting biofilm samples. All implant sites for sampling were then isolated using sterilized cotton rolls. Supra-gingival plaque sample was identified and removed using sterilized cotton pellets. Following this, the isolated area was then air-dried. The microbial samples were then collected by gentle insertion of fine-tipped sterilized paper points in the following sites for 10 seconds. Sites for collection of plaque sample included a) Gingival sulcus located mesially as well as distally to site of placement of the implant and b) Alveolar ridge at an edentulous site.

In edentulous ridge areas, paper points were then placed within the vestibule and alveolar ridge. Paper points that were soaked in Gingival Crevicular Fluid were then placed within sterile transport vials containing 1 ml of anaerobic medium and were then sent to a laboratory. Bacterial culture media used in this study included blood agar, Kanamycin blood agar, and 'Kanamycin‑vancomycin' containing blood agar.

SAMPLE COLLECTION METHOD

Biofilm samples were collected as per the following protocol a) pre-operatively i.e., before prescribing antibiotics (baseline sample, S1), b) 1-day post-operatively or first stage surgery (S2), c) sample collected at the time of removal of sutures removal (seven to ten days post-operatively or S3, d) Sample collected at two weeks or S4, (5) at time of second-stage surgery or S5, e) sample collected two days following the placement of abutment or S6, f) sample collected on the day of placement of prosthesis or S7; g) sample collected two days following the placement of a prosthesis or S8; g) sample collected at one month recall or follow up; S9 and (10) at two months recall follow-up, S10.

RESULTS AND OBSERVATIONS

All collected samples were then tested for estimating microbial growth of the following pathogenic micro-organisms: Streptococcus, A. actinomycetemcomitans, P. intermedia, P. gingivalis and F. nucleatum. The values of the microbiological analysis of both the study groups i.e., with the immediate and delayed placement of implants) have been shown in Tables 1 and 2.

Table 1: Counts of various micro-organisms detected during different stages of placement of delayed implants

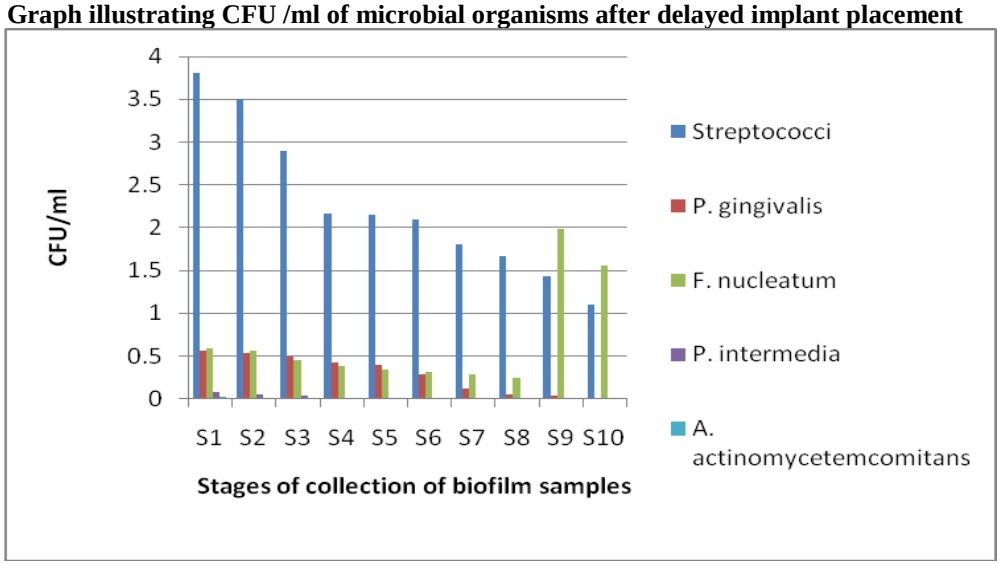
Micro-organisms (CFU /ml)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Streptococci	3.8 $X10^3$	3.5 $X10^3$	2.9 $X10^3$	2.17 $X10^3$	2.15 $X10^3$	2.09 $X10^3$	1.8 $X10^3$	1.67 $X10^3$	1.43 $X10^3$	1.1 $X10^3$
P. gingivalis	0.56 $X10^3$	0.53 $X10^3$	0.49 $X10^3$	0.43 $X10^3$	0.39 $X10^3$	0.28 $X10^3$	0.12 $X10^3$	0.05 $X10^3$	0.04 $X10^3$	0.01 $X10^3$
F. nucleatum	0.59 $X10^3$	0.56 $X10^3$	0.45 $X10^3$	0.39 $X10^3$	0.35 $X10^3$	0.32 $X10^3$	0.29 $X10^3$	0.25 $X10^3$	1.98 $X10^3$	1.56 $X10^3$
P. intermedia	0.08 $X10^3$	0.06 $X10^3$	0.04 $X10^3$	0.01 $X10^3$	0.001 $X10^3$	0	0	0	0	0
A. actinomycetemcomitans	0.02 $X10^3$	0.01 $X10^3$	0	0	0	0	0	0	0	0

Table 2: Microbial counts observed during different stages of placement of immediate implants

Micro-organisms (CFU /ml)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Streptococci	3.9 $X10^3$	3.6 $X10^3$	3.0 $X10^3$	2.09 $X10^3$	2.01 $X10^3$	2.00 $X10^3$	1.23 $X10^3$	1.01 $X10^3$	0.45 $X10^3$	0.23 $X10^3$
P. gingivalis	0.49 $X10^3$	0.42 $X10^3$	0.37 $X10^3$	0.30 $X10^3$	0.21 $X10^3$	0.14 $X10^3$	0.1 $X10^3$	0.05 $X10^3$	0	0
F. nucleatum	0.45 $X10^3$	0.39 $X10^3$	0.34 $X10^3$	0.25 $X10^3$	0.19 $X10^3$	0.02 $X10^3$	0.01 $X10^3$	0	0	0
P. intermedia	0.02 $X10^3$	0.01 $X10^3$	0.01 $X10^3$	0	0	0	0	0	0	0
A. actinomycetemcomitans	0.01 $X10^3$	0	0	0	0	0	0	0	0	0

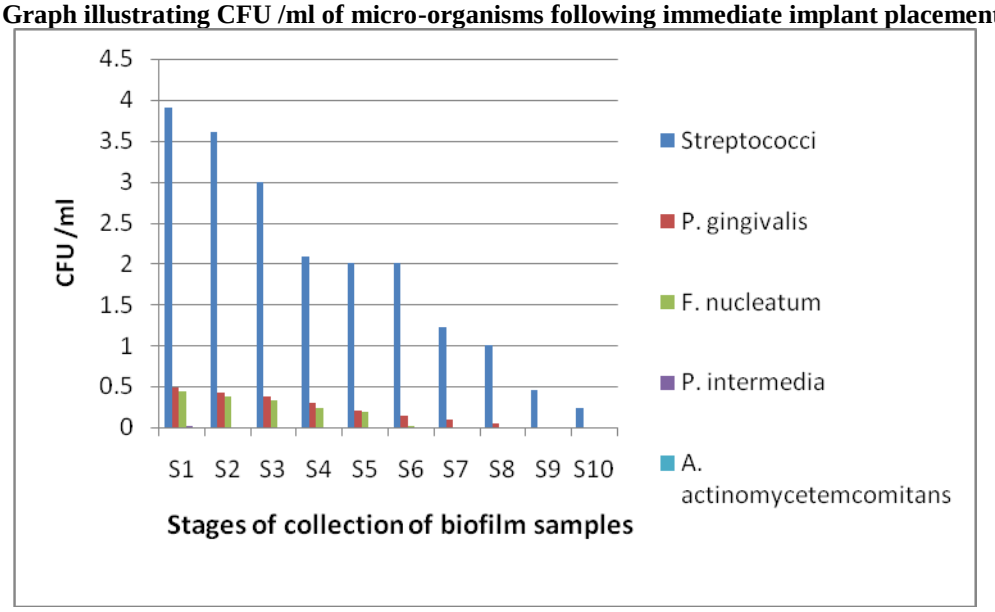
Appropriate statistical analysis tests were employed for the analysis of obtained data and for calculating obtained results. Comparison of mean concentrations of various micro-organisms in group 1 with the delayed placement of implants demonstrated statistical significance (Kruskal–Wallis = 41.248; P-value < 0.001).
Streptococcal organisms were present in consistently higher titers as these are normal commensal organisms within the oral cavity while other microorganisms like *Porphyromonas gingivalis*, *Fusobacterium*, *Prevotella intermedia*, and *A. actinomycetemcomitans* demonstrated relatively lower titers. (Table and Graph 1)

Graph 1: Graph illustrating CFU /ml of microbial organisms after delayed implant placement



On comparing mean concentrations of identified micro-organisms in the immediately placed implant a statistical significance was observed (Kruskal–Wallis = 40.169 and P < 0.001).
The presence of pathogenic micro-organisms, for example, *P. gingivalis* and *Fusobacterium* which had pathogenic potential was of greater importance. (Table and graph 2)

Graph 2: Graph illustrating CFU /ml of micro-organisms following immediate implant placement



However, on comparison of mean ranks of Streptococci, *F. nucleatum*, *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcometans* in delayed as well as immediately placed implant groups a statistically non-significant result was obtained.

DISCUSSION

Osseointegration of dental implants demonstrates the unique opportunity for observing the earliest colonization of the bacterial population and also, for estimating the time that is required for establishing complex microbiological flora as it deals with initiation from a surface that is free of bacterial cells.[8]

The long-term success associated with immediate implants is found to be comparable to the delayed placement of implants as numerous factors are considered important for determining long time success as well as the failure of the implant systems which include occlusal loading forces, type of implant material, method of surgical placement and acceptance by the host. However, there is only limited knowledge regarding the importance of sub-gingival bacterial colonization surrounding implants and their harmful effects over peri-implant-related tissues.

Peri-implantitis may be found to independently affect differently placed dental implants within the same oral cavity, however, it is still not clear if the microbiota is different at each of the sites or is variable at different sites. [9,10]

There is very limited information available on the presence of microbiological flora in tissues surrounding an immediately placed dental implant. In the current study, peri‑implant colonization of microbes and their virulence in subjects with both immediately placed and delayed placed implants were studied. Papaioannou et al in 1997 by making use of 'phase‑contrast' microscopy along with DNA probes studied the prevalence of periodontal pathogenic organisms in partial edentulous and fully edentulous subjects who had a history of periodontal diseases. It was observed that the microbial profiles had similarities around teeth as well as dental implants with equal depth of periodontal pockets indicating that these pockets surrounding teeth might be serving as a reservoir for these periodontal disease-related pathogens. [11]

In the present study, it was found that placement of implants either delayed or when they are immediately placed do not cause alteration in the microflora of the oral cavity. Thus, one must understand that maintaining periodontal tissue health is important for preventing peri-implantitis. Although, it is important for identifying these micro-organisms for understanding their level of pathogenicity as well as monitoring the patients for good clinical outcomes.

However, in contrast to our study findings, Blank et al in 2021 could not isolate P. gingivalis from biofilm samples collected around implants. [12]

Hiremath et al in 2020 reported that Streptococci showed consistent presence from the initial level. Micro-organisms such as Porphyromonas gingivalis as well as Fusobacterium were present after placement of either delayed or immediate types of implants and had pathogenic potential importance. [13]

Lefaurie et al (2017) in their results demonstrated that *T. forsythia* was the most common red-complex organism in peri-implantitis sites which was followed by *T. denticola* and *Porphyromonas gingivalis*. [7]

The structural composition of peri‑implant associated microflora has similarity to that of microflora present in pockets surrounding all-natural teeth that is an obligate type of ecological microbial niche for few of the oral micro-biota. [14]

Leonhardt et al (1999) on comparing microbiological flora found adjacent to healthy oral implants with those around implants wherein peri-implantitis has

been observed reported that 60 % of dental implants with peri-implantitis were infected with following microorganisms such as *P. gingivalis, P.nigressence, P. intermedia along with A. actinomycetemcomitans* while 55 % of peri-implantitis cases had the presence of *Staphylococcus spp., Candida spp*. and *enteric* organisms while no organisms were detected in the implants with healthy surrounding tissues. [15]

CONCLUSION

The peri-implant disease develops many years after placement of implants hence, a regular follow-up for monitoring coupled with assessing peri‑implant micro-flora was important for a good prognosis. Hence, it is recommended that longitudinal studies must be done out using a large-sized sample for arriving at highly specific conclusions on the presence of specific micro-organisms during and after implant placement.

REFERENCES

1. Apse P, Zarb GA, Schmitt A, Lewis DW. The longitudinal effectiveness of osseointegrated dental implants. The Toronto Study: peri-implant mucosal response. Int J Periodontics Restorative Dent 1991; 11(2): 94-111.
2. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B et al. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991;2(2): 81-90.
3. Lang NP, Berglundh T. Peri-implant diseases: where are we now?Consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol 2011; 38 Suppl 11: 178-81.
4. Madi M, Alagl AS. The Effect of Different Implant Surfaces and Photodynamic Therapy on Periodontopathic Bacteria Using TaqMan PCR Assay following Peri-Implantitis Treatment in Dog Model. Biomed Res Intern 2018; ttps://doi.org/10.1155/2018/7570105.
5. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol 2008;35(8 Suppl): 286-91.
6. Nassar H, Kantarci A, Van Dyke TE. Diabetic periodontitis: a model for activated innate immunity and impaired resolution of inflammation. Periodontol 2000 2007;43: 233-44.
7. Lafaurie GI, Sabogal M A, Castillo D M . Microbiome and microbial biofilm profiles of peri-implantitis: A systematic Review. *J Periodontol 2017;* 88 (10):1066–89,
8. Flanagan D. Implant Placement in Failed Endodontic Sites: A Review. J Oral Implantol 2016;23(2)224-30.
9. Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra‑osseous anchorage of dental prostheses. Experimental studies. Scand J Plast Reconstr Surg 1969;3:81‑100.
10. Ghensi P, Manghi P, Zolfo M, Armanini F, Pasolli E, Bolzan M et al. Strong oral plaque microbiome signatures for dental implant diseases identified by strain-resolution metagenomics. Npj Biofilms and Microbiomes 2020;6:47-59.
11. Papaioannou W, Quirynen M, Van Steenberghe D. The influence of periodontitis on the subgingival flora around implants in partially edentulous patients. Clin Oral Implants Res 1996;7:405‑9.
12. Blank E, Grischke J, Winkel A, Eberhard J, Kommerein N , Doll K, Yang I et al. Evaluation of biofilm colonization on multi‑part dental implants in a rat model. BMC Oral Health 2021; 21:313-25.
13. Hiremath KG, Patil VN, Ruttonji Z, Kusugal P, Sushma KM, Astagi P. A comparative evaluation of changes in microbial flora in delayed and immediate placed implants: An *in vivo* study. J Dent Implant 2020;10:16-21.
14. Tripodakis AP, NAkou M. Microbiologic evaluation of compromised periodontal sites before and after immediate intrasocket implant placement. Int J Periodontics Restorative Dent 2011;31:e109-17.
15. Leonhardt A, Renvert S, Dahlen G. Microbial findings at failing implants. Clin Oral Implants Res 1999; 10(5):339-45.