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IN VIVO EVALUATION OF MICROBIAL CHANGES IN DELAYED VERSUS IMMEDIATE IMPLANT PLACEMENT

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ABSTRACT

Background: Peri-implant microbial colonisation is one of the most critical determinants of the success of osseointegration of implants and long-term outcomes. This in vivo study seeks to determine and compare qualitative and quantitative changes that occur in peri-implant microflora during immediate and delayed implant placement.

Materials and Methods: A total of 30 systemically healthy participants who needed single tooth extraction and implant placement were enrolled and equally allocated into two groups. Group A (immediate placement of an implant), and Group B – a delayed implant placement 12 weeks after surgery. Peri-implant sulcus was sampled at the start (pre-placing), the first month, and three months after placement. For this, sterile paper points were utilized. Cultivation and identification were done by culture-based methods together with colony-forming unit (CFU) counts for *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. Clinical measures such as plaque index and gingival index, and peri-implant probing depth were also documented.

Results: Group A exhibited a higher mean CFU count for P. gingivalis (2.8×10^5CFU/mL) than Group B (1.2×10^5CFU/mL). The S. mutans colonization at three months was comparable for both groups. There was a statistically significant ($p<0.05$) increase in anaerobic species in Group A. The clinical parameters did not exceed the limits in both groups. However, slightly better gingival scores and reduced inflammation were demonstrated in Group B.

Conclusion: One of the possible consequences of the immediate implant placement might be a higher level of colonization of pathogenic anaerobic bacteria during the preceding period of healing in comparison with delayed implant placement. Postponed placement may promote more optimal processes of soft tissue healing and microbial stabilization. Monitoring of per-implant microbial profiles is critical in the beginning stages of implant therapy.

Keywords: Immediate implant, delayed implant, peri-implant microbiota, *P. gingivalis*, microflora, in vivo study, CFU count.

1. INTRODUCTION

Dental implants have transformed restorative dentistry as a reliable and predictable therapy for replacing missing teeth. There has been a lot of interest and clinical debate about the timing of implant placement: immediate versus delayed.

Immediate implant placement implies the insertion of the implant fixture into the extraction socket during the same surgical visit, and the delayed placement is

performed after a healing period of several weeks to months after tooth extraction[1]. Each of these approaches has its merits and limitations in the aspects of esthetics, soft tissue healing, and preservation of peri-implant bone.

Establishment and stability of the peri-implant microbial environment are one of critical factors that determine the success of dental implants. The colonization of the implant surfaces by the oral microbial flora, in particular by pathogenic anaerobic microbes, can be the stimulus for the peri-implant inflammation and imperil osseointegration[2–4]. Studies have shown that the microbial pattern around the implant is similar to that observed in the natural periodontal sulcus and often dominated by *Porphyromonas gingivalis, Fusobacterium nucleatum, and Aggregatibacter actinomycetemcomitans*[5]. These bacteria are highly associated with peri-implantitis and implant failure[6].

Prompt implant insertion is beneficial in terms of retaining treatment duration and saving alveolar bone, yet, in compromising the surgical site and having poor soft-tissue coverage for the initial period of healing early on, there is the increased risk for microbial contamination[7]. On the other hand, delayed placement of the implant allows for initial soft and hard tissue healing, which may decrease microbial load and encode a more stable peri-implant situation[8,9].

With a large body of literature on implant microbiology, very few studies of a similar nature have been carried out in vivo to determine the microflora change associated with immediate and delayed implants. Insight into evidence of changes in the microbial dynamics in the implants installed with various intervals of time is essential to optimize the clinical protocols and enhance the long-term prognosis of implants. This research aims to assess and compare qualitative and quantitative alterations in implant microflora in immediate and delayed implant placement using the culture-based method of microbial analysis.

2. MATERIAL AND METHODS

This in vivo comparative study was conducted on 30 patients aged between 25 and 55 years, who required a single tooth replacement in the posterior region of the mandible or maxilla. All participants were systemically healthy, non-smokers, and demonstrated good oral hygiene.

Study Design and Group Allocation

Participants were randomly assigned to two groups of 15 patients each:

- Group A: Immediate implant placement immediately after tooth extraction.
- Group B: Delayed implant placement performed 12 weeks post-extraction after adequate soft tissue and bone healing.

Atraumatic extraction was performed with the use of periotomes and forceps to avoid damage to the surrounding bone. In Group A, implants were positioned in the fresh extraction socket in the same surgical visit. In Group B, the time to heal and therefore the time to the extraction was 3 months, and implants were placed after. All implants were inserted under local anesthesia, according to a standard surgical protocol. Bone grafts and membrane barriers were not used between the two implants to prevent confounding factors. The postoperative instructions and antibiotics for five days were given to the patients.

The peri-implant sulcular fluid was drawn using sterile paper points at three intervals of time. Baseline (pre-operative), 1 month, and 3 months after the implantation. Paper points were inserted into the peri-implant sulcus for 30 seconds prior to being put into sterile transport media right away.

Samples were grown on selective media to detect and count *Streptococcus mutans, Porphyromonas gingivalis, and Fusobacterium nucleatum*. Incubation was carried out under conditions lacking oxygen for 48-72 hours. CFUs per mL were calculated and recorded for every bacterium.

Some of the clinical measures, such as Plaque Index (PI), Gingival Index (GI), and peri-implant Probing Depth (PD), were recorded at baseline, after 1 month, and after 3 months. All clinical assessments were conducted by a calibrated examiner to eliminate variability.

SPSS version 25.0 statistical package was used to analyze the data. Intergroup comparisons were conducted with the aid of an independent t-test, while intra-group comparisons over the time points were analyzed with the aid of repeated measures ANOVA. Statistical significance was assumed at a p-value < 0.05.

3. RESULTS

A total of 30 patients (15 in each group) completed the study without any implant failure or postoperative complications. The demographic distribution was comparable between the two groups, with a mean age of 38.4 ± 6.2 years in Group A and 39.1 ± 5.8 years in Group B.

Microbial Analysis

The mean colony-forming units (CFU/mL) for *Porphyromonas gingivalis, Fusobacterium nucleatum,* and *Streptococcus mutans* at baseline, 1 month, and 3 months are shown in **Table 1**. At 1 month and 3 months, *P. gingivalis* and *F. nucleatum* levels were significantly higher in Group A (immediate placement) compared to Group B (delayed placement) ($p < 0.05$). *S. mutans* levels remained relatively stable across both groups with no statistically significant differences.

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Table 1. Mean CFU/mL of Key Microorganisms at Different Time Intervals in Both Groups

Bacterial Species	Time Point	Group A (Immediate)	Group B (Delayed)	p-value
P. gingivalis	Baseline	$1.1 \times 10^5 \pm 0.2$	$1.0 \times 10^5 \pm 0.3$	0.612
	1 Month	$2.4 \times 10^5 \pm 0.5$	$1.3 \times 10^5 \pm 0.4$	0.032*
	3 Months	$2.8 \times 10^5 \pm 0.4$	$1.2 \times 10^5 \pm 0.3$	0.018*
F. nucleatum	Baseline	$0.9 \times 10^5 \pm 0.1$	$0.8 \times 10^5 \pm 0.2$	0.408
	1 Month	$2.0 \times 10^5 \pm 0.3$	$1.0 \times 10^5 \pm 0.2$	0.029*
	3 Months	$2.5 \times 10^5 \pm 0.5$	$1.1 \times 10^5 \pm 0.3$	0.015*
S. mutans	Baseline	$1.3 \times 10^5 \pm 0.3$	$1.2 \times 10^5 \pm 0.4$	0.676
	1 Month	$1.4 \times 10^5 \pm 0.2$	$1.3 \times 10^5 \pm 0.3$	0.543
	3 Months	$1.4 \times 10^5 \pm 0.3$	$1.2 \times 10^5 \pm 0.2$	0.482

*Significant at $p < 0.05$

Clinical Parameters

Clinical parameters such as Plaque Index (PI), Gingival Index (GI), and Probing Depth (PD) are summarized in **Table 2.** At 3 months, Group B showed significantly lower GI and PD values compared to Group A ($p < 0.05$), while plaque scores remained statistically similar between the two groups.

Table 2. Comparison of Clinical Parameters Between Immediate and Delayed Implant Groups

Parameter	Time Point	Group A (Immediate)	Group B (Delayed)	p-value
Plaque Index	Baseline	0.82 ± 0.11	0.80 ± 0.13	0.743
	3 Months	0.95 ± 0.09	0.89 ± 0.08	0.356
Gingival Index	Baseline	1.02 ± 0.14	0.98 ± 0.12	0.624
	3 Months	1.24 ± 0.10	0.96 ± 0.11	0.041*
Probing Depth	Baseline	2.2 ± 0.3 mm	2.1 ± 0.4 mm	0.487
	3 Months	2.6 ± 0.4 mm	2.1 ± 0.3 mm	0.038*

*Significant at $p < 0.05$

As shown in **Table 1**, microbial colonization by anaerobic pathogens was significantly higher in the immediate implant group at both follow-up points. This was further supported by the clinical findings in **Table 2**, which indicated increased inflammation and probing depth in the same group.

4. DISCUSSION

The current in vivo study sought to assess and compare the peri-implant microbial changes between the immediate and delayed implant placement programs. The results indicated that there were significantly more colonization of the pathogenic anaerobic bacteria, such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, in the immediate implant placement compared to the delayed implant placement at 1 month and 3 months post-placement. Based on these findings, one can conclude on a greater number of microbes, which in turn may have led to the increased risk for peri-implant inflammation in the immediate implant protocols during the early healing phase.

The initial colonization of the implant surfaces by the bacteria from the oral cavity is, therefore, a key factor governing the sustained long-term success of osseointegration and implant stability[1]. Immediate implantation into fresh extraction sockets can lead to premature exposure to biofilms in the oral cavities before the soft tissue maturation, which will hence favor the propagation of periopathogenic species[2,3]. As opposed to it, delayed implant placement permits the surgical site initial healing, which possibly can reduce microbial contamination and enhance more favorable tissue integration[5,6].

Results of our study are consistent with earlier works that indicated increased numbers of anaerobic bacteria in immediately placed implants over delayed protocols[7,8]. For example, the increased prevalence of *P. gingivalis* and *F. nucleatum* in the crevicular fluid of immediate implants was observed significantly by Devides SL et al.[10], implying delayed healing responses and more susceptibility to microbes. The same trends were present in the present study, where the immediate group exhibited nearly double counts of the CFU of such pathogens compared to the delayed group, at the 3-month evaluation.

The fairly constant levels of Streptococcus mutans in both groups could mean that facultative anaerobes do not have significant changes based on implant timing. This observation corresponds to findings from Lee KH et al., who revealed that the early microbiota associated with implants is essentially commensal streptococci before the shift to anaerobic colonization[11].

Some of the clinical parameters, like Plaque Index, Gingival Index, and Probing Depth, are adjunct indicators of health status around implants. Our delayed group exhibited better gingival health and fewer records of probing depths after 3 months, and the same was consistent with fewer anaerobic bacterial counts. These clinical outcomes reflect the corresponding microbiological results and confirm a hypothesis that delayed implants constitute a more facilitative environment for early healing[12–14].

There are numerous mechanisms that could affect microbial colonization and peri-implant health, such as surgical technique, oral hygiene of patients, surface characteristics of implants, and perioperative care[15]. It is worth mentioning that in the current study, all implant plastic surgeries were performed under standardized protocols and with the same surgeon, meaning we have reduced operator-dependent variability. Limitations are the relatively short follow-up, as well as the use of culture-based microbiology, which could miss the unculturable species that some advanced molecular techniques could unveil[16,17].

However, clinically, the implication of this study is valid. The greater number of microbial challenges recorded in immediate implant placements calls for selective choice of patients, practices of high infection control measures, and postoperative monitoring. In active infection or poor quality of soft tissue, a delayed surgical approach could result in optimal outcomes.

Greater cohorts, longer durations of follow-up, and implementation of next-generation sequencing analysis, thus, can provide further information on the dynamics of the microbial population around implants in regard to implant timing and their positive or negative contribution to the health of peri-implant tissues[18].

5. CONCLUSION

Results of this in vivo study show that immediate implant placement entails a greater early colonization of anaerobic periopathogenic bacteria than as compared to delayed implant placement. Peri-implant tissues and microbial control were better during the first stages of healing for those with a delay in implants. Such results highlight the need for suitable case selection, microbial control, and rigid hygiene procedures during immediate implant surgeries to optimize clinical outcomes and long-term implant prosperity.

DECLARATIONS:

Ethical approval and consent to participate

Not Applicable

Availability of data and material –All data generated or analyzed during this study are included in the published article.

Competing interest – The authors declare that there are no competing interests.

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