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Original Article

Microbial evaluation of implant surface – a clinical study comparing submerged, non-submerged and immediately loaded implants

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Aim: The aim of the present study was to evaluate the microbial contamination in internal and external walls of cone morse implant walls. **Methods:** Eleven patients with edentulous mandibular posterior area were selected to received dental implants, divided into groups: submerged (S), non-submerged (NS), and immediately loaded (IL). Microbiological evaluations (microorganisms' number, aerobic and anaerobic colony forming units (CFU) number and microorganisms' qualification) were divided into internal and external collection of the implant walls, at different stages: T0 (surgical procedure), T2 (suture removal), T4 (reopening S group), T6 (suture removal S group), and T8 (abutment placement in S and NS). All data were submitted to statistical analyses, with confidence level of 0.05. **Results:** There was difference in number of microorganisms observed over time within the same group ($p < 0.05$). A difference was observed in CFU when evaluated within the same group over time ($p < 0.05$), except for the IL group. In internal collection, a predominance of non-formation of microorganisms was observed at T0 in all groups, while formation of Gram-positive Diplococci and Gram-positive Bacilli was observed at T8 ($p>0.05$). In external collection, an increase in number of microorganisms was observed at T0. **Conclusion:** There was no difference in microbial contamination among the evaluated groups. The microorganism's colonization changed over time.

Keywords: Dental implants. Surgery, oral. Actinobacteria.

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Introduction

Dental implants are currently a safe and predictable reality for patients that wish to rehabilitate their oral health[1]. There are two surgical protocols for dental implants insertion: one-stage surgery or non-submerged implants; two-stage surgery or submerged implants. In the first technique, the implant is placed so that the soft tissue flap remains around the coronal portion of the implant body or healing abutment. In the second surgical technique, the implant is placed at or below the alveolar crest level and the soft tissue is closed around the implant. Then, after a suitable period, a second procedure is performed to expose the implant platform and install a healing or a final abutment[2]. During the initial phase of osseointegration, most implant systems recommend that the implant remain submerged[3-5].

There are several reasons for the use of submerged implants, including minimizing the risk of infection, reducing vertical bone level loss and reducing the risk of excessive early loading[6]. A radiographically randomized clinical trial conducted by Giacomel et al., in 2017[7], compared changes occurred in marginal bone level between immediately loaded implants (IL) delayed loaded non-submerged implants (NS) and delayed loaded submerged implants (S). The different protocols used for implant placement and loading did not show statistically significant differences in bone level changes around the implants.

Since the implant is composed of an endosseous part that connects it to the prosthetic part, even if these structures are closely attached, a microgap of up to 50µ may exist between them[8]. Within this context, it is essential that the connection between abutment and implant is as effective as possible in order to prevent or reduce the risk of bacterial colonization, since the greater the proximity between the microgap and the alveolar bone, the greater the expected bone loss[9].

Although there is lack of in vivo studies comparing bacteria in submerged, non-submerged and immediately loaded implants, outcomes from a clinical study suggested that all the external hexagon, double internal hexagon, internal hexagon with external collar and conical connection contaminated after 5 years of functional loading[10]. Progressive colonization by periodontopathogenic bacteria in the internal cavities of two-piece implants has been previously described[11]. Besides, submerged implants with screw-retained structures are considered to be more susceptible to pathogenic microflora than non-submerged implants[12].

Therefore, the aim of the present study was to evaluate quantitatively the microbial contamination in internal and external walls of cone morse implant walls in submerged (S), non-submerged (NS) and immediately loaded (IL) implants at different stages.

Material and Methods

Ethical approval was obtained from the Ethics Research Committee of the University, under protocol number 69244216.5.0000.0093.

Study Population

The inclusion criteria were adult patients with at least three missing teeth that should be mandibular premolars and/or mandibular molars. Patients who entered the study

received oral hygiene instructions. In addition, all patients in the study were submitted to prophylaxis every three months. Thus, we were able to submit the same patient to submerged, non-submerged and immediately loaded implants, under the same oral and systemic conditions, decreasing bias.

Patients were excluded from the study if any of the following criteria was present: (1) Smoking, pregnancy or use of bisphosphonates (2) Uncontrolled systemic disorders; (3) Parafunction; (4) Poor oral hygiene; (5) The presence of active periodontal disease or caries; (6) A need for bone reconstruction before implant insertion.

Patients were consecutively selected according to the inclusion and exclusion criteria, and to the period planned for patient recruitment. Sample size calculation was not performed but was based on a previous study of our group[7] that evaluated the same three different approaches: submerged, non-submerged and immediately loaded implants in marginal bone level changes.

Anamnesis and Clinical Examination

In the first evaluation, a complete anamnesis and physical/clinical examination, definition of the treatment plan and oral hygiene instructions were performed. An occlusal evaluation to determine the prosthetic space available was also carried out. In this case, the occlusal height and the possibility of rehabilitation after implant placement were verified. The patients were maintained, until the end of the research, in an adequate plaque control regimen. All treatment steps were performed and registered by the same calibrated examiner with experience in Implantology (FF). In addition, all patients had a mandible tomography performed on the i-CAT Classic (Imaging Sciences International, Hatfield, Pennsylvania, USA). With this examination, the size of the implant to be installed in the region was defined.

Surgical Procedures

All patients received three dental implants divided into three groups according to the installation protocol. The submerged group (S) implants received a delayed loading and remained submerged until the reopening phase and subsequent prosthetic rehabilitation. The non-submerged group (NS) received delayed loading with non-submerged implants until the prosthetic rehabilitation phase. The immediately loaded group (IL) implants received immediate loading without abutment removal for prosthetic rehabilitation.

Surgery began for all implant groups with a mid-crestal incision in the edentulous area. The same milling sequence was used for the confection of the bone window and implant placement and consisted of initiating drilling with a 2.0 mm-diameter spear drill, followed by a 2.0 mm-diameter helical drill and by a milling cutter specific for 3.5 mm-diameter SW Morse implants (SIN – Implant System, São Paulo, Brazil). All implants were placed 1 mm below the bone crest level. After implant insertion, the final torque was evaluated. Patients were advised regarding postoperative care and mechanical control of bacterial plaque. To control postoperative pain, patients were instructed to use ibuprofen 600 mg every eight hours for three to five days. Amoxicillin 500 mg was also prescribed every eight hours for seven days for patients not allergic to penicillin. Sutures were removed seven days after implant placement.

Collection and Microbiological Analysis

Microbial collection was performed in two ways: internal and external. The internal collection was held in the internal part of the surrounding walls of the implant and the external collection in the external part of the implant walls, always at a point located in the buccal surface.

The internal collection analysis was conducted in the following stages: T0 - Internal collection in S Group, NS Group and IL Group on the day of surgery. In the IL group, a single internal collection was performed since the abutment was not removed after placement (Figure 1A); T4 - After a period of 90 days, a new collection was executed in the S Group, on the day of reopening and insertion of the healing screw; T8 - After the removal of the healing screw and before the insertion of the final abutment, a new internal collection was carried out in S Group and NS Group. The final abutment was then placed, and a provisional acrylic resin restoration was cemented.

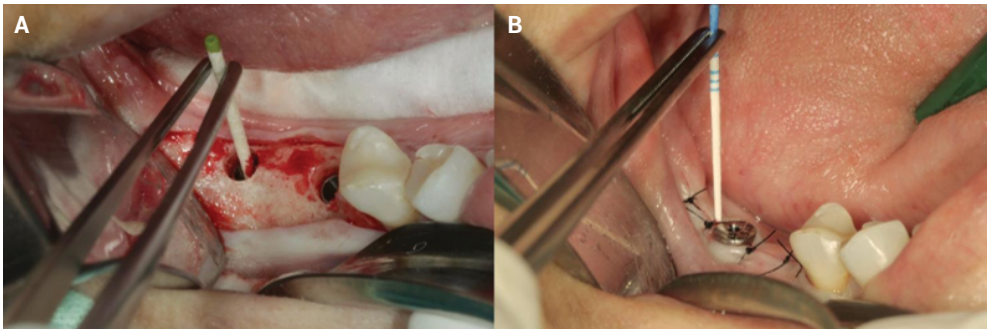

Figure 1. (A): Internal collection at T0, NS group. **(B):** External collection at T0, located on the periimplant groove, NS group.

The external collection analysis was conducted in the following stages: T0 - For IL Group and NS Group on the day of implant insertion, in which material was collected in the abutment transmucosal area in the IL Group and in the transmucosal area of the healing screw in the NS Group. At this stage, there was no collection in S Group implants, as they remained submerged (Figure 1B); T2 - Material collection in the abutment transmucosal area in the IL Group and in the transmucosal area of the healing screw in the NS Group, on the day of suture removal; T4 - On the day of reopening in the S Group, the material was collected in the transmucosal area of the healing screw; T6 - Material collection around the transmucosal area in the S group, on the day of suture removal; T8 - The material was collected around the transmucosal area in NS group and S group after placement of the final abutment.

Evaluation of Colony Forming Units (CFU)

Qualitative evaluations were performed by inoculating the material in petri dishes with blood agar culture. Bacterial proliferation was evaluated after a period of 24 hours at a temperature of approximately 37°C. For the CFU quantification, the criterion

used was "no growth", "countable growth" and "uncountable growth" as expressed: No growth = < 1 CFU; Countable growth - 1 a 250 CFU and Uncountable growth - If it is not possible to count the number of colonies on the plate, as they are too numerous to count (> 250 CFU).

Spectrophotometric Analysis of Optical Density

To perform the analysis of optical density of all groups, the material was collected in T0, T2, T4, T6 and T8 and read immediately. In addition, a new reading occurred after a period of 24 hours (T1, T3, T5, T7 and T9) in which the collection was incubated at a temperature of approximately 37°C.

Gram Staining

The gram staining process started by covering the entire surface of the plate on which the smear was performed with crystal violet (purple dye), for two minutes, draining the excess. The plate was again covered with lugol solution (mordant) for one minute. Once it was rinsed with distilled water, acetone-alcohol solution was dripped over it for about fifteen seconds, followed by a new wash. Again, the plate was covered with carbol fuchsin, followed by rinsing and drying. Once the plate was completely dry, a drop of entellan was applied on the side where the smear layer was performed and covered with a coverslip, waiting for the entellan to dry completely. After a period of 24 hours, the plate was analyzed using a microscope. After staining, the plates were classified according to the presence of Gram-negative Bacilli, Gram-positive Bacilli, Gram-negative Diplococci, Gram-positive Diplococci, Gram-negative Cocci, Gram-positive Cocci and funghi. The amount of growth was quantified as no growth (-), little growth (+), average growth (++) and substantial growth (+++) which is considered in values in percentage. no growth (-) corresponds to 0%, little growth (+) corresponds to 1 – 33.33%, average growth (++) corresponds to 33.34 to 66.66% and substantial growth (+++) corresponds to 66.67 to 100%.

Statistical analysis

The data were tabulated and submitted to analysis using SPSS software for Windows 24.0 (SPSS Inc., Chicago, Illinois, USA). The data were submitted to normality test. After the test, the numerical variables were considered non-parametric, being cataloged by the median, minimum and maximum. The CFUs were evaluated as an ordinal variable. Statistically significant differences were considered when $p < 0.05$.

Results

Of the eleven patients evaluated, the majority of the sample was female (63.6%) and Caucasian (90.9%). Mean age was 49.91 years (±10.23). Table 1 presents the results of spectrophotometric analysis of optical density for all groups over time, both in internal and external collections. It is possible to observe that there was a statistically significant difference in the number of microorganisms when the same group was assessed over time ($p < 0.05$). In T1, T3, T5, T7, and T9 there was greater bacterial growth than in other phases since the other stage correspond to the analysis performed after 24 hours of culture of the collection.

Table 1. Spectrophotometric analysis of optical density evaluating the submerged (S), non-submerged (NS) and immediately loaded (IL) groups at the different stages

Collection	Groups	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	p-value (stage)
		Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Internal	S	0.03[b] (0.01–0.32)	1.15[a] (0.53–1.59)	-	-	0.72[ab] (0.12–0.82)	1.54[a] (0.81–2.11)	-	-	0.00[b] (0.03–0.09)	1.28[a] (1.06–1.72)	< 0.001
	NS	0.036[ab] (0.01–0.23)	0.89[c] (0.13–1.51)	-	-	-	-	-	-	0.46[a] (0.01–0.20)	1.22[b] (0.82–1.44)	< 0.001
	IL	0.04[a] (0.01–0.64)	1.24[b] (0.17–1.58)	-	-	-	-	-	-	-	-	0.005
External	S	-	-	-	-	0.44[a] (0.10–0.59)	1.08[a] (0.36–2.21)	0.04[b] (0.01–0.10)	1.28[a] (0.91–1.55)	0.03[bc] (0.01–0.05)	1.28[a] (1.06–1.72)	< 0.001
	NS	0.15[a] (0.09–0.34)	1.54[ab] (0.97–1.71)	0.25[a] (0.09-0.36)	1.83[b] (1.31-2.25)	-	-	-	-	0.03[c] (0.02–0.15)	1.35[b] (1.02–1.70)	< 0.001
	IL	0.22[a] (0.13–0.70)	1.74[b] (1.15–1.86)	0.22[a] (0.14–0.72)	1.95[b] (1.05-2.26)	-	-	-	-	-	-	< 0.001

Note: Friedmann test for three or more categories, Wilcoxon test for two categories; Different letters mean statistical significance at $p < 0.05$

Table 2 shows the comparison among groups of the results obtained in the spectrophotometric analysis of optical density at each stage. There was no statistically significant difference between groups ($p > 0.05$).

Table 2. Spectrophotometric analysis of optical density comparing the studied groups at each stage

Collection	Stages	Submerged Group	Non-submerged Group	Immediately Loaded Group	p-value
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Internal	T0	0.04 (0.01–0.32)	0.04 (0.01–0.30)	0.07 (0.01–0.64)	0.896
	T1	1.15 (0.30–1.59)	1.17 (0.13–1.56)	1.25 (0.17–1.58)	0.929
	T8	0.05 (0.02–0.09)	0.05 (0.01–0.20)	-	1
	T9	1.34 (1.06–1.95)	1.19 (0.82–1.56)	-	0.104
External	T0	-	0.15 (0.02–0.36)	0.22 (0.10–0.70)	0.06
	T1	-	1.53 (0.99–1.71)	1.62 (0.66–1.86)	0.695
	T2	-	0.18 (0.09–0.36)	0.22 (0.14–0.76)	0.292
	T3	-	1.83 (1.31–2.25)	1.73 (1.05–2.26)	0.794
	T8	0.03 (0.01–0.05)	0.03 (0.02–0.33)	-	0.179
	T9	1.42 (1.06–1.72)	1.35 (1.02–1.70)	-	0.479

Note: Kruskall-Wallis test for three or more categories, Mann-Whitney test for 2 categories; significance level of 0.05. The external collection was not performed in S group at T2 and T3 since implants remained submerged in these stages.

The comparison among the different groups of implants (S, NS, and IL) regarding the number of aerobic and anaerobic CFU formed at T0, T2, and T8 did not present a statistically significant difference (Table 3).

Table 3. Comparison among the Submerged, Non-Submerged and Immediately Loaded groups regarding the aerobic and anaerobic CFU count.

		T0 p value	T2 p value	T8 p value
Internal	Aerobic (S, NS, IL)	0.423	-	1
	Anaerobic (S, NS, IL)	0.378	-	1
External	Aerobic (S, NS, IL)	0.365	0.748	0.730
	Anaerobic (S, NS, IL)	0.562	0.748	1

Note: Kruskall-Wallis test for three or more categories, Mann-Whitney test for 2 categories; significance level of 0.05. The internal collection could not be compared between groups at T2 since implants in S group remained submerged in this stage.

The intragroup comparison showed a statistically significant difference in the number of aerobic and anaerobic CFU formed over time in S group and NS group in the internal collection and in NS group in the external collection ($p < 0.05$) (Table 4).

Table 4. Quantification of aerobic and anaerobic colony forming units (CFU) within the submerged (S), non-submerged (NS) and immediately loaded (IL) groups over time

Collection	Groups	Stages	Aerobic CFU (n)				Stages	Anaerobic CFU (n)			
			No Growth (n)	Countable (n)	Uncountable (n)	p-value		No Growth (n)	Countable (n)	Uncountable (n)	p-value
Internal	S	T0[a]	5	6	0	>0.001	T0[a]	8	3	0	0.02
		T4[b]	-	3	6		T4[bc]	2	3	4	
		T8[bc]	-	-	9		T8[b]	-	-	9	
	NS	T0	7	4	0	0.03	T0	9	2	0	0.02
		T8	-	-	9		T8	-	-	9	
	IL	T0	8	3	0	-	T0	6	5	0	-
External	S	T4	1	2	6	0.05	T4	-	-	9	1
		T6	-	-	9		T6	-	-	9	
		T8	-	-	9		T8	-	-	9	
	NS	T0[a]	5	2	4	0.02	T0[a]	5	3	3	0.02
		T2[ab]	-	4	7		T2[ab]	-	-	9	
		T8[b]	-	-	9		T8[b]	-	-	9	
	IL	T0	3	3	5	0.10	T0	2	5	4	0.18
		T2	-	2	9		T2	-	4	7	

Note: Kruskall-Wallis test for three or more categories, Mann-Whitney test for 2 categories; significance level of 0.05. Different letters mean statistical significance at $p < 0.05$

Table 5 compares the number of aerobic and anaerobic CFU formed within groups at the different stages, according to the type of microorganism identified by gram staining.

Table 5. Classification of the different types of microorganisms present within groups at the different stages

Collection	Groups	Microorganisms		T0	T2	T4	T6	T8
Internal	Submerged	Non-formation	Aerobic	+++	X	-	X	-
			Anaerobic	+++	X	+	X	-
		G+ Diplococci	Aerobic	+++	X	++	X	+++
			Anaerobic	++	X	++	X	+++
		G+ Bacilli	Aerobic	-	X	+++	X	+++
			Anaerobic	-	X	+++	X	+
	Non-Submerged	Non-formation	Aerobic	+++	X	X	X	-
			Anaerobic	+++	X	X	X	-
		G+ Diplococci	Aerobic	+++	X	X	X	+++
			Anaerobic	+	X	X	X	+++
		G- Diplococci	Aerobic	-	X	X	X	-
			Anaerobic	-	X	X	X	-
		G+ Bacilli	Aerobic	-	X	X	X	++
			Anaerobic	-	X	X	X	+
	Immediately Loaded	Non-formation	Aerobic	+++	X	X	X	X
			Anaerobic	+++	X	X	X	X
		Diplococo G+	Aerobic	++	X	X	X	X
			Anaerobic	++	X	X	X	X
		Funghi	Aerobic	+	X	X	X	X
			Anaerobic	+	X	X	X	X
External	Submerged	Non-formation	Aerobic	-	X	+	-	-
			Anaerobic	-	X	-	-	-
		G+ Diplococci	Aerobic	-	X	++	+++	+++
			Anaerobic	-	X	+++	+++	+++
		G- Diplococci	Aerobic	-	X	-	+	-
			Anaerobic	-	X	-	+	
		G+ Bacilli	Aerobic	-	X	+++	+	++
			Anaerobic	-	X	+++	+	+
		G- Bacilli	Aerobic	-	X	+	+	-
			Anaerobic	-	X	-	-	-
		G+ Cocci	Aerobic	-	X	-	+	-
			Anaerobic	-	X	-	+	-
		G- Cocci	Aerobic	-	X	-	+	-
			Anaerobic	-	X	-	+	-
		Funghi	Aerobic	-	X	++	+++	-
			Anaerobic	-	X	-	-	+

Continue

Continuation								
External	Non-Submerged	Non-formation	Aerobic	+++	-	X	X	+
			Anaerobic	+++	-	X	X	+
		G+ Diplococci	Aerobic	+++	+++	X	X	+++
			Anaerobic	+++	+++	X	X	+++
		G- Diplococci	Aerobic	-	+	X	X	-
			Anaerobic	-	+	X	X	-
		G+ Bacilli	Aerobic	+	++	X	X	+++
			Anaerobic	-	-	X	X	+
		G+ Cocci	Aerobic	-	+	X	X	-
			Anaerobic	-	-	X	X	-
		G- Cocci	Aerobic	-	+	X	X	-
			Anaerobic	-	-	X	X	-
		Funghi	Aerobic	-	+	X	X	-
			Anaerobic	-	-	X	X	+
	Immediately Loaded	Non-formation	Aerobic	++	-	X	X	X
			Anaerobic	+	-	X	X	X
		G+ Diplococci	Aerobic	+++	+++	X	X	X
			Anaerobic	+++	+++	X	X	X
		G- Diplococci	Aerobic	-	+	X	X	X
			Anaerobic	-	-	X	X	X
		G+ Bacilli	Aerobic	-	+++	X	X	X
			Anaerobic	-	++	X	X	X
		Funghi	Aerobic	-	-	X	X	X
			Anaerobic	-	+	X	X	X

X – Collection was not performed; - no growth; + CFU little growth; ++ CFU average growth; +++ CFU substantial growth.

Discussion

The concern of implant clinicians regarding the best choice of implant for a specific area is a constant point of discussion. One of the main topics that guide the decision is based on the choice of whether to use submerged, non-submerged or immediately loaded implants. Therefore, the main objective of the present study was to investigate if there is a difference among the studied groups regarding bacterial contamination externally or internally to dental implants. The microbiota in the oral environment determines to a large extent the composition of the flora developing around implants[13]. It has been hypothesized that submerged implants may present a lower risk of infection, less vertical bone loss and lower risk of implant overloading[3-5,14]. Furthermore, it has been hypothesized that immediately loaded implants would present lower internal microbial contamination when compared to other implants.

As for the assessment of the same implant over time, the present study found a significant difference in microbial contamination both by spectrophotometric analysis

and by CFU count. One fact that draws attention is that, in some specific stages it was demonstrated greater bacterial contamination than in others. Gaps and hollow spaces within the implant system, for example the gap between implant and abutment in the two-part implant system, may provide a bacterial reservoir causing or maintaining inflammation. The bacterial spectrum involved is similar to that found in periodontitis[15]. In general, in our study there was more microorganism growth in the external collections than in the internal collections, except for the group of submerged implants (S group), in which values remained similar. This may be explained by the existence of a microgap between the abutment and the implant, which is an area where there is a higher concentration of microorganisms. Thus, the greater the gap between the implant and the abutment, the greater the microbial contamination in the area, leading to greater bone loss[8-9,16-18].

In the present study, no difference in microbial contamination was found among the different groups of implants, either by spectrophotometric analysis or by CFU count, contradicting the initial hypothesis in which it was thought that immediately loaded implants would be advantageous since they would present lower microbial contamination. There are no studies in the literature comparing the three types of implants evaluated up until the writing of this study. However, some interesting studies have been previously carried out assessing microbial contamination, mainly comparing different abutment designs. In a study conducted by de Moraes Rego et al.[16] significantly higher counts of *A. gerencseriae* and *S. constellatus* were found in implants placed at the supracrestal level compared to the ones placed at the bone level. No relation was found between the installation level of dental implants and peri-implant bone remodeling. Peruzetto et al.[19], in 2016 evaluated the bacterial seal at the implant-abutment interface using two Morse taper implant models, by in vitro microbiological analysis. The authors concluded that both tapered components failed to provide adequate sealing to bacterial leakage, although the indexed type components showed a superior seal compared with non-indexed components.

Another important issue of the study refers to the CFU quantification for each of the groups in each one of the stages. It was possible to observe that there was greater microbial contamination within the same group of implants over time, and that there were more CFU externally than internally. It is known that contamination of internal implant and suprastructure components has shown considerable great biodiversity, indicating bacterial leakage along the implant-abutment interface, abutment-prosthesis interface, and restorative margins. Cosyn et al.[17] compared microbiologically the peri-implant sulcus to these internal components on implants with no clinical signs of peri-implantitis and in function for many years. The authors concluded intra-coronal compartments of screw-retained fixed restorations were heavily contaminated. The restorative margin may have been the principal pathway for bacterial leakage. Contamination of abutment screws most likely occurred from the peri-implant sulcus via the implant-abutment interface and abutment-prosthesis interface.

In order to qualify the microorganisms present, gram staining was used. Thereby making it possible to observe the presence of gram-positive and gram-negative

microorganisms. Normal microbiota of healthy implants includes Gram-positive rods and cocci. Peri-implantitis is caused by pathogens, especially Gram-negative bacteria like *Veillonella sp.* and *spirochetes*, including *Treponemadenticola*[20,21]. In this study, in the internal collection of the implant wall, it was observed a predominance of non-formation of microorganisms at T0 in all groups, with formation of Gram-positive Diplococci and Gram-positive Bacilli at T8. On the other hand, in the external collection, an increase in the number of microorganisms was observed at T0, when compared to the internal collection. Fungi were also predominant in the external collection.

One limitation of the study is that no characterization of the evaluated bacteria was carried out. With our results it is possible to know the morphology of the bacterium and if it positive or negative. We cannot say, however, whether the quantification of the bacteria indicates a greater predisposition to peri-implantitis and/or bone loss. Moreover, it is important to consider that the bacteria found could be part of the normal flora and the hygiene of each patient could influence the results during the collection of biological material. We recommend a more detailed analysis in future studies.

Another important point is that at T4 (Reopening S group), the external collection was not performed in the NS group since the healing process of the tissue around the healing screw prevented the insertion of the paper cone in that region.

Also, it is important to highlight the choice for the use of antibiotics in the postoperative period. We chose to use this type of medication in order to reduce the possibility of infectious processes related to the procedure. However, it should be emphasized that the primary objective of the study is to compare the three groups and all of them were subject to the same effects of the antibiotic in the seven-day period, which we believe does not make the results unfeasible. There was also no control over the systemic drugs used by patients during the collection of biological material. However, it is worth mentioning that patients who take systemic medications continued the intake during all evaluations, therefore, we understand that this does not interfere with the results, as the comparisons are performed on the same individual.

Based on the present results, it can be stated that regardless the insertion of submerged or non-submerged implants, no difference is expected in their microbial contamination. Thus, considering the reduction of surgical stages, the insertion of non-submerged implants, may present as an advantage if the basic principles of osseointegration are respected, such as good primary stability and sufficient bone quantity[4].

Finally, it is possible to infer that there is no difference concerning the amount of microbial contamination among the studied groups, only for microorganism's colonization over time.

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