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Comparative Analysis of the Presence of Staphylococcus Epidermidis in the Implant-Abutment Junction Zone in Different Dental Implant Models

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Abstract

The aim of this study was to investigate the amount of Staphylococcus epidermidis present at the implant-abutment interface of two different brands of dental implants. Twenty implants and 20 straight internal connection abutments of these brands were analyzed by immersing them in blood agar in Petri dishes and evaluating the DNA of the colonies at the interface. Using a saliva sample dipped in a Staphylococcus epidermidis strain, the number of microorganisms entering the implant-abutment interface was determined. Brand B1 exhibited a lower presence of Staphylococcus epidermidis (range 0-2000 CFU/ml) compared to brand C1, which presented a higher quantity (range 12000-19000 CFU/ml). It was evident that the presence of Staphylococcus epidermidis at the implant-abutment interface of B1 (average 800 CFU/ml) was lower than in C1 (1560 CFU/ml). These results indicate a moderate presence in C1 and a slight presence in B1, and it was statistically determined that the adhesion and quantity of Staphylococcus epidermidis depend on the colonized surfaces ($p < 0.05$).

Keywords: *Staphylococcus epidermidis, dental implants, DNA, Petri dishes*

1. Introduction

The replacement of lost teeth through the use of analogous structures that are integrated into oral tissues constitutes an important clinical advance in the field of dentistry This therapeutic modality, known as dental implants, has revolutionized the oral restorative approach by offering a highly effective and durable alternative to rehabilitate the masticatory and aesthetic function of patients (1).

Dental implants have proven their effectiveness for approximately 45 years, based on the key principle of osseointegration. This phenomenon is defined as the direct connection, both functional and structural, between the surface of the implant subjected to functional load and the living and organized bone. Successful osseointegration requires the interaction of various factors, including tissue remodelling and repair, as well as healing capacity. These biological and cellular processes play a critical role in forming a stable and long-lasting bond between the implant and the surrounding bone tissue. Tissue remodelling allows continuous adaptation of the bone around the implant, promoting its stability and resistance to functional loads.

The healing ability ensures a controlled inflammatory response and the formation of a blood clot at the implant insertion site, which in turn initiates the cascade of events leading to bone regeneration and the formation of a strong bond between the implant and bone. The deep understanding of these biological processes has driven the design and optimization of dental implants, allowing to achieve satisfactory and predictable clinical results in the field of oral rehabilitation (2,16,17).

On the other hand, it is important to note that osseointegration in itself is not enough to guarantee the success of treatment with dental implants. In this sense, considerable effort has been devoted to the improvement of both the manufacture of implants in mechanical and aesthetic terms this comprehensive approach seeks to improve not only the ability to integrate with the bone, but also the functionality and aesthetic appearance of the implants.

The continuous improvement of clinical processes has also been a fundamental objective to achieve optimal results. Advances in technology and research have made it possible to develop implants with more sophisticated designs and high-quality materials, which are better adapted to the individual needs of each patient. In addition, clinical protocols have been refined to optimize the precise and safe placement of implants, minimizing risks and maximizing treatment effectiveness. This comprehensive and multidisciplinary approach, which covers both technical and clinical aspects, has contributed significantly to the continuous improvement of results in dental implantology and has allowed to provide solutions of higher quality and durability to patients (3,18,19).

In order to achieve the aesthetics and health of the tissues that are around the implant, the union between the hard and soft tissues is essential, the soft peri-implant tissues are divided into oral keratinized epithelium, which is continued with the union epithelium, and the connective tissue which contains abundant collagen fibers (3).

The peri-implant tissue mentioned above, is dependent in its lower part on a hard tissue scaffolding, which is the bone, it is permanently remodelled, under normal circumstances, but this balance can be affected and lead to the loss of it around the implant, determined by several factors, among which we find: The type of implant used, the presence of the implant-abutment interface, the existence of microorganisms, whether or not we find keratinized tissue, the quality of soft tissue around the implant and the surgical technique. After osseointegration has been achieved, the selected prosthetic connection will determine the stability of the implant (4).

The survival of the implant and the presence of microorganisms at the interface of the implant and its pillar within the rehabilitation treatment will be altered by the stability achieved in this aspect. One of the disadvantages cited when using materials formed by titanium in the manufacture of trans mucous elements is in aesthetic aspect since its colour can be appreciated through soft tissues (5).

2. Materials And Methods

Type and Design of Research

The present study is framed as a comparative, experimental and in vitro research, aimed at analyzing and evaluating various variables related to the presence of Staphylococcus epidermidis at the implant-abutment interface.

The main objective of this study is to examine the differences in the amount and behaviour of Staphylococcus epidermidis at the implant-abutment interface between two commercial brands of dental implants. This choice of research design allows for a direct and accurate comparison between implants, making it easier to identify potential differences in bacterial colonization and behavior of Staphylococcus epidermidis.

The in vitro experimental methodology was selected to recreate controlled conditions that would allow a precise and systematic analysis of the samples. This guarantees the validity and reliability of the results obtained, since possible confusing variables or external interferences are eliminated. By using this methodology, more precise cause and effect relationships can be established and the direct influence of the variables studied on the presence and behavior of Staphylococcus epidermidis in the implant-abutment interface can be determined.

Through the comparison of key variables, such as the number of bacterial colonies, biofilm formation and antimicrobial resistance, this study seeks to provide a significant contribution to scientific knowledge in the field of dental implants and oral microbiology. The results obtained may have both clinical implications, by helping health professionals to make informed decisions about the use of different commercial brands of implants, and therapeutic, by providing relevant information for the development of strategies for the prevention and control of bacterial colonization at the implant-abutment interface.

In summary, the present study adopts a comparative, experimental and in vitro approach to analyze the variables related to the presence of Staphylococcus epidermidis at the implant-abutment interface. The in vitro experimental methodology allows a precise and systematic evaluation of the samples, guaranteeing the validity and reliability of the results obtained. The findings of this study may contribute to scientific knowledge in the field of dental implants and oral microbiology, with both clinical and therapeutic implications.

Study Population

The study population consists of a universe of 20 implants and 20 straight internal connection abutments from two different commercial houses these implants and abutments were carefully chosen to be part of the analysis and evaluation within the framework of this research, in order to obtain relevant and significant results on the presence of Staphylococcus epidermidis at the implant-abutment interface, The inclusion of implants and abutments from two different commercial houses provides a broader and more realistic perspective of the diversity existing in the field of dental implants, and allows direct comparison between the products of both brands in terms of bacterial colonization at the interface. This rigorous and representative research approach guarantees the robustness of the results obtained and contributes to the advancement of knowledge in the field of dental implantology.

Sample

An exhaustive analysis of a sample consisting of 20 implants and 20 straight internal connection abutments was carried out, which were carefully selected from the trademarks C1 and B1, recognized in the field of dentistry. In order to investigate the presence of Staphylococcus epidermidis at the implant-abutment interface, these specimens were immersed in Petri dishes containing blood agar, and incubated for a period of 72 hours under controlled conditions.

Subsequently, a thorough analysis of the DNA of the colonies present at the interface of each implant was carried out, using advanced molecular techniques. The main objective of this analysis was to examine both the presence and behavior of Staphylococcus epidermidis in relation to the different trademarks studied, in order to provide an accurate and meaningful comparison between them. This rigorous and detailed methodological approach allowed to obtain high quality scientific data, providing a solid basis for the analysis and interpretation of the results obtained in the context of the research.

Inclusion Criteria

Lyophilized strain of Staphylococcus epidermidis obtained at the National Institute of Public Health Research INSPI Leopoldo Izquieta Pérez. ATCC 25175.

Implants obtained in commercial houses B1 and C1. Diameter 4 mm. Indexed morse connection. Pillars obtained in the respective commercial houses B1 and C1, of 1.5 mm metallic tape (ucla), for indexed morse connection.

Exclusion Criteria

Contamination of the implant when handling Lack of torque in the abutment implant connection screw. Excess torque in the abutment implant connection screw. Contamination in prosthetic attachments when handling. At the time of removing the prosthetic attachment that contaminates the abutment implant connection. Tape of different size other than 1.5 mm. Pillar for any connection type other than indexed morse.

Data Collection Management and Method

For this research, we worked with implants acquired respectively in commercial houses with prosthetic attachments. Selective growth media blood agar, thioglycolate and mannitol biochemical tests were supplied by the Netlab clinical laboratory. The strain of Staphylococcus epidermidis was supplied by the Izquieta Pérez Institute which was transferred to the Stuart medium. The experimental part was carried out in the clinical laboratory "Villa Lab" in the city of Quito. The quality of the strains and the culture medium were kept in refrigeration between 2 to 8° C. so that the results were reliable immediately after reception (4). It was taken out of refrigeration and waited a while for the strain and agar to be acclimated. Stuart medium was seeded to selective agar, following a striating process, then taken to the incubator "globe" 37 ° C which was controlled by temperature meters calibrated for 24 to 72 hours (6,20,21).

After incubation, the macroscopic morphological characteristics obtained in the blood agar were observed, characteristics that coincided with the genus of Staphylococus which is a very resistant microorganism, represented by non-motile Gram-positive cocci growing in colonies of approximately 1.2 millimetres in diameter (4). A gram stain was performed in which it was expected to visualize gram-positive cocci arranged in clusters to ensure we were working with the genus Staphylococcus, the catalase test was performed which consists of taking a colony in question, taking it to an object cover and placing hydrogen peroxide, observing detachment of gas bubbles, Indicating a positive catalase result, when performing the coagulase test, that is, homogenizing a colony of bacteria plus plasma, the formation of clot indicative of a negative coagulase test is not visualized (7,22).

To ensure the presence of the species of epidermidis was performed the biochemical test of mannitol, which is a pink medium where positivity is reflected in yellow, where positivism is not reflected means that it is negative and it is confirmed that in the genus and species it is positive for Staphylococcus epidermidis. Once the bacterium was identified, the investigation began with the implants, using the thioglycolate nutritive broth (liquid medium), the implants were placed, each commercial house was represented with a number to avoid conflict, commercial house 1 and commercial house 2 with their respective metal-based pillars at a torque of 30 N prior to colonization with Staphylococcus epidermidis, the implants were handled with biosafety standards using titanium tweezers for manipulation, thus avoiding contamination of other bacteria, were placed in the tubes with thioglycolate and a portion of Staphylococcus epidermidis colony was previously seeded in the rabbit blood agar with a calibrated handle in the thioglycolate nutrient broth and immediately taken to incubation at 37 ° C. for 24 h to 72 h.; At 24 hours of incubation with thioglycolate proceeded to take from the portion of the interface implant - pillar of each commercial house a sample of Staphylococcus epidermidis, performing a sowing on rabbit blood agar to incubate at 37 ° for 24 h to observe how much bacteria grew in that time, performing the same procedure at 48 and 72 hours.

After the analysis to evaluate the growth or not of the implant-pillar interface, the count of colony forming units was carried out and it was verified if the bacterium is Staphylococcus epidermidis, after planting the gender and species tests were performed again following the standardized microbiological protocols of the case.

Once the procedure was performed in the laboratory "Villa Lab" the colonized implants were taken and placed in a sterile Petri dish to be transferred to the University of the Armed Forces E.S.P.E where the sample was prepared according to the protocol to be able to observe in the Scanning Electron Microscope at an increase of 10000 on a scale of 100 microns.

Figure 1 Implant abutment interface "B1"

Figure 2 Culture of the C1 abutment implant interface

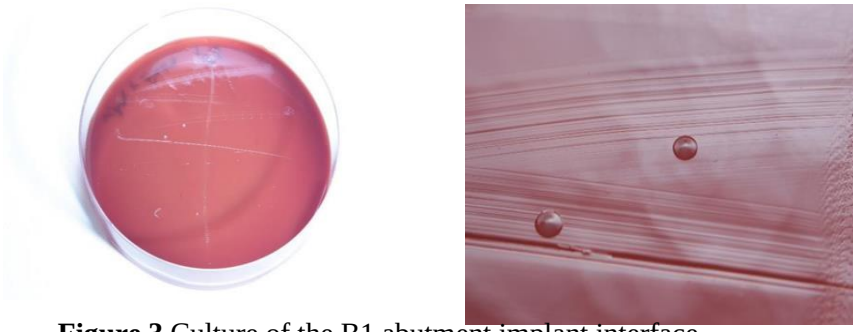

Figure 3 Culture of the B1 abutment implant interface

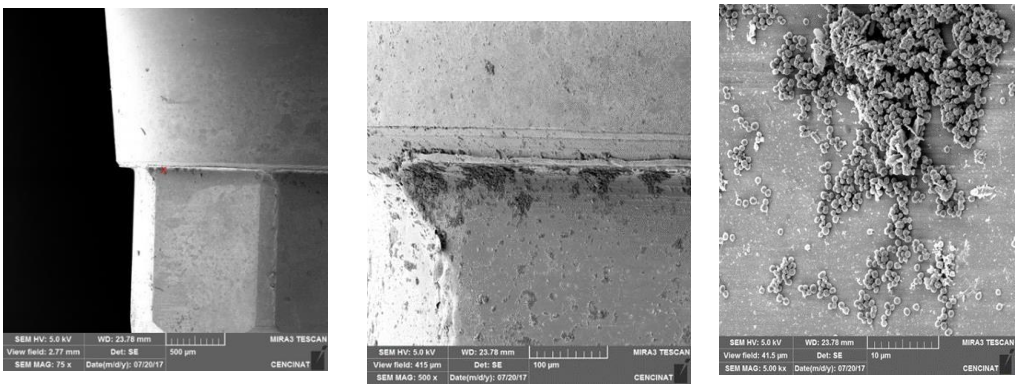

Figure 4 Electron microscopy SEM implant C1

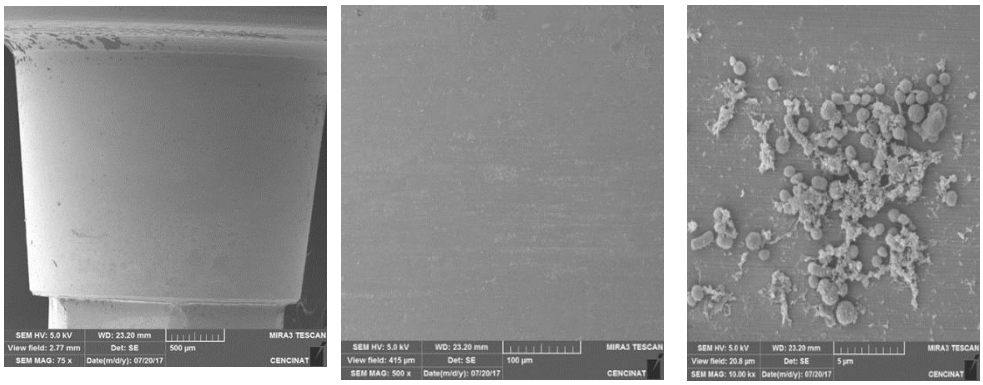

Figure 5 Electron microcopy SEM implant B1

3. Results and Discussion

In the implant-abutment interface of B1 exhibits the lowest presence of Staphylococcus epidermidis between a range of 0-2000 CFU / ml, while the implant-abutment interface of Conexao presents the highest amount of microorganism in a range of 12000 – 19000 CFU / ml. In addition, the presence of Staphylococcus epidermidis in the implant-pillar interface of Bionnovation with an average of 800 CFU/ml, is lower than the presence of Staphylococcus epidermidis in the implant-pillar interface of Conexao with 1,560 CFU/ml.

Board 1. Results of the presence of Staphylococcus epidermidis in the implant-pillar interface of the different brands.

Samples	B1 (CFU/ml)	C1 (CFU/ml)
1	1.000	17.000
2	1.000	19.000
3	0	15.000
4	0	18.000
5	1.000	14.000
6	1.000	16.000
7	0	12.000
8	2.000	15.000
9	0	14.000
10	2.000	16.000
Average	800	15.600

Board 2. Descriptive results of the T-Student: Mean of colony forming units.

Samples	Stocking	N	Standard deviation	Standard error mean	
Par 1	C1 (CFU/ml)	15600,00	10	2065,591	653,197
	B1 (CFU/ml)	800,00	10	788,811	249,444

Board 3. T-Student test: Statistical difference between group C1 and B1

Samples	Stocking	Standard deviation	Standard error mean	95% confidence interval of difference		t	Gl	Sig. (bilateral)	
				Inferior	Superior				
Part 1	B (CFU/ml) - A (CFU/ml)	14800,00	2043,96	646,36	13337,84	16262, 16	22,90	9	,000

Implant-associated infections remain one of the biggest challenges faced by patients and doctors after surgery. Bacteria that attach to implanted devices can colonize the surface and develop a resistant biofilm, which in turn leads to chronic infection that is resistant to host defense mechanisms and antimicrobial medication (8,9).

Based on the above, the present research is based, where it was demonstrated that the adhesion, formation and quantity of Staphylococcus epidermidis is dependent on the surfaces through an in vitro

study, in this case of the implant-pillar interface of C1, which presented the largest amount of microorganism in a range of 12000 – 19000 CFU / ml than the implant-abutment interface of B1, It was also confirmed that there was a statistically significant difference when contrasting the means of the Staphylococcus epidermidis Colony Forming Unit that adhered to the two types of implants used in the research.

In view of the fact that when reviewing the literature on the subject, no study was found in the country where the susceptibility of the implant-abutment interface to the adherence of Staphylococcus epidermidis is compared. For this reason, reference would be made to the research of Rabelo et al. (10), in which they studied the leakage of Streptococcus sanguis in several implants-pillar of different brands, among which was the C1, demonstrating that in the internal hexagonal Morse implant C1 there was a bacterial contamination of Streptococcus sanguis ATCC10556 62.50%.

This shows that the implant-pillar C1 interface is prone to contamination with microorganisms, which can cause various peri-implant diseases, because the implants are subjected to functional loads there is fluid exchange between internal and external medium that increases bacterial infiltration in the peri-implant area and therefore there is a greater risk of bacterial colonization by not having an adequate seal between the implant-abutment interface[CITATION Ass09 \ l 3082 \m Pia011 \m Tab15]. Aloise et al. (2010) (12), have confirmed that microfiltration occurs in both directions, from the internal parts of the implants to the external environment and vice versa.

Reported measures to prevent or minimize bacterial contamination of the implant-abutment interface, such as the use of sealing materials, decontamination of the internal implant cavity, use of shape memory alloy and different connection geometries, have not been successful (13,23).

The colonization of bacteria at the implantation interface depends on factors such as the accuracy at the implant-abutment interface of different implant systems and their marginal adjustment, the closing torque values also alter the sealing capacity of the abutments (11,14).

In addition, the study by Assunção et al. (4), indicate that in the restoration of dental implants the presence of microgaps between the implant-abutment can cause microbial leaks or a storage of the same in this interface, being able to penetrate through a space as small as 10 μm. According to Lakha et al. (15) microbial penetration through the microgaps invariably exists from the implant-abutment interface. This minimal opening has proven to be a potential source of microbial infiltration and peri-implantitis leading to implant failure as it offers a welcoming environment for bacterial colonization.

Although conical connections have demonstrated better sealability, microinterval invariably exists at the interface, so it can be stated that no connection has completely eliminated the formation of micro-gaps or led to a sterile environment within the implant connection. This information is consistent with what was reported in this research.

4. Conclusion

After performing the detailed analysis, it was determined that in the implant-abutment interface of the C1 mark, a constant presence of Staphylococcus epidermidis was observed in an average range of 15600 CFU / ml, indicating a moderate bacterial presence. On the other hand, in the implant-abutment interface of the B1 brand, a presence of Staphylococcus epidermidis was identified in a medium range of 800 CFU/ml, suggesting mild bacterial contamination.

In addition, a marked difference of 14800 CFU/ml was found between both brands, with a higher concentration of Staphylococcus epidermidis forming units at the implant-abutment interface of C1. The results obtained were supported by a statistical analysis that significantly demonstrated that the adhesion, formation and amount of Staphylococcus epidermidis are influenced by the characteristics of the colonized surfaces ($p < 0.05$).

These findings highlight the importance of considering differences in implant surface properties when assessing bacterial colonization and may have significant implications in the development of strategies for the prevention and treatment of dental implant-related infections.

It is strongly recommended to use the implant-abutment interface of the B1 brand during dental implant procedures, due to its lower adhesion, formation and amount of Staphylococcus epidermidis

bacteria compared to the implant-abutment interface of other brands studied. This finding is relevant for both health professionals and manufacturers, as it evidences the dependence of the adhesion, formation and quantity of Staphylococcus epidermidis in relation to the characteristics of the colonized surfaces.

It is crucial to note that microbial colonization in the microgaps and on the inside of the implant can lead to soft tissue inflammation, which in turn can lead to implant failure. For future studies, it is recommended to consider the size of the interface between the implant and the abutment, in order to establish a more precise association with the amount of bacteria that can adhere in the microgaps. These findings will contribute to a better understanding of the factors influencing bacterial colonization at the implant-abutment interface, and will allow the development of more effective strategies to prevent infectious complications and ensure the long-term success of dental implants.

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