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Evaluation of Bacterial Contamination Along Implant-Abutment Interface

Sugandha Gupta[1], Nitin Modi [2], Vaibhav Awinashe [3], Mukul Shetty [4], Debasruti Naik [5], Minal Vaibhav Awinashe[6]

[1]*Assistant Professor, Department of Prosthodontics, Dental College, Azamgarh, UP, India.*

[2]*Department of Prosthodontics, Crown & Bridge and Implantology, Vyas Dental College and Hospital, Jodhpur, Rajasthan, India.*

[3]*Department of Prosthodontics, College of Dentistry in Ar Rass Qassim University, Buraidah, Kingdom of Saudi Arabia*

[4]*Department of Orthodontics and Dentofacial Orthopedics, AB Shetty Memorial Institute of Dental Sciences, NITTE (Deemed to be University), Derlakatte, Karnataka, India.*

[5]*Department of Public Health Dentistry, Institute of Dental Sciences, Shiksha O Anusandhan University, Bhubaneswar, Odisha, India.*

[6]*Department of Oral Surgery and Diagnostic Sciences, College of Dentistry, Qassim University, Buraidah, Kingdom of Saudi Arabia.*

Abstract

Fluid infiltration is made easier by the tiny gap (microgap) that exists between the implant and abutment.The objective of this research was to assess the relationship between bacterial contamination and interface size along the implant-abutment contact. Twenty internal-hex and twenty external-hex brand name implants were used. Under aseptic conditions, the implants were housed in a box and immediately underwent internal inoculation with 0.3 µl of the *Streptococcus sanguis* ATCC10556 bacteria. The abutment was torqued to 25 Ncm for the external hex and 15 Ncm for the internal hex. The system was then included in an Eppendorf control for 15 seconds and left in the control for 25 days. While the Eppendorf controls were cultured in blood agar to examine the colonies that grew, the implants were taken out and evaluated using a scanning electron microscope. Utilising the Chi-squared, Mann-Whitney and Kruskal-Wallis tests, the data were examined, with a significance level of $p<0.05$ being taken into account. Five implants were not included since they were likely contaminated externally. There were no significant differences seen between the various systems, with microspaces measuring up to 82.6 µm in the external-hex implants and 48.7 µm in the internal-hex implants ($p>0.05$). The majority of the contamination was found in the external hex implants, and there were statistically considerable variations between the several hex systems made by the same company. To the best of our knowledge, there was no correlation between *Streptococcus sanguis* bacterial contamination and the size of the implant-abutment interface.

Keywords: Bacterial, contamination, dental implant, interface

Full-length article **Corresponding Author, e-mail:doctornitinmodi@gmail.com*

1. Introduction

At the implant-abutment (I-A) interface, instability or misfitting have been linked to implant therapy failures [1,2]. One of the main issues in building two-stage implant systems is microbial leakage at the implant-abutment interface. Between the implant and the abutment, spaces and cavities develop that allow microbes to seep out. An important contributing factor to peri-implant inflammatory reactions is this leaking. The precision of the fit between the fixture and abutment determines the degree of bacterial colonisation between the implants and abutments [3,4]. Inflammatory reactions to peri-implant soft tissue can be brought on by bacteria and their byproducts [5]. In close proximity to bone, the existence of a micro-gap, and consequently a bacterial reservoir, may contribute to the onset of inflammation in the peri-implant tissues and bone loss [6].Numerous endeavours to achieve a more robust bond between the implant fixture and the abutment base have been examined. The most often used connectors are usually combinations of internal and external shapes, such as hexagonal, conical (Morse taper), or both. It has been reported that the internal implant abutment connection facilitates fluid infiltration more than other joints [7]. One problem linked to implant loss is peri-implantitis [8].This study aims to assess the microbiological leakage of two distinct implant abutment connections in vitro.

2. Materials and Methods

This investigation was carried out in the Oral Implantology and Prosthodontics Department with consent from the institutional ethical committee. After taking into account the inclusion and exclusion criteria, the study was completed.A total of forty implants were utilised, sourced from the following brands: Adin, Israel®, BIOMET3i ®, INP® (Sao Paulo, Brazil), and BioHorizons ®. Each implant included a UCL-A abutment, and 20 of the implants included internal and exterior hex systems fabricated of commercially pure titanium. Eight groups, consisting of five implants each, were created based on the brand and hex type of the sample.

2.1. Microbiological evaluation

In compliance with the manufacturer's recommendations, the implants and the abutment system were completely sterilised. Due to the operator's requirement for sensitivity, the stages of the experimental model were carried out at five different times on various days. Each phase comprised eight implants, with two implants from each group used at each analysis point until a total of 40 implants were analysed under identical conditions.The bacteria used in the analysis were *Streptococcus sanguis* ATCC (24 10556 SS-A-TCC), which was bred in BHI culture (Brain Heart Infusion, Biolife, Milan, Italy) and incubated at 37°C for 24 hours in a bacteriological incubator (Biomatic, Porto Alegre, RS, Brazil). The bacteria were activated 24 hours prior to each experiment using 100 µl of the previously defrosted strain.

After that, the experiment was carried out utilising sterile materials inside an aseptic box. Next, 0.3 µl of *Streptococcus sanguis* ATCC10556 bacteria were added to BHI solution contaminated with SS-ATCC using a precision pipette.Next, in accordance with the manufacturer's recommendations, the abutments were torqued with 15 Ncm for internal-hex implants and 25 Ncm for external-hex implants. The system was introduced in a (control) Eppendorf containing 1.5 ml of BHI culture media for 20 seconds in a perfectly sterile environment to confirm any potential external contamination; thereafter, the implant was withdrawn and placed in another Eppendorf for 25 days. During the initial Eppendorf reading, which took place 24 hours after the experiment began; the colour of the medium was checked to ensure that there was no contamination. If any contamination was found, the implant was taken out of the study due to the possibility of external contamination or sterilisation system failure.

Upon completion of the experiment, the contaminated material in the Eppendorf was taken out in order to prepare the slides for Gramm staining and to breed the microbe on a Petri dish containing blood agar for the catalyse test, which verified the morphology and traits of the contaminating bacteria.

2.2. Scanning electron microscopic assessment

Using a scanning electron microscope (SEM) with magnifications ranging from 25 to 2500x, measurements were collected between the implant and the abutment after the implants were removed and allowed to dry for two hours.Utilising the Chi-squared, Mann-Whitney and Kruskal-Wallis tests in Stata v. 9.0, the data were examined, with a significance level of $p<0.05$ being taken into account for statistical analysis.

3. Results

The levels of contamination found in various systems varied, with the Adin, Israel ® HE, and BioHorizons ® HI implants being the only ones devoid of bacterial contamination. In the microbiological contamination analysis, no significant differences were found across the various firms, but a statistically significant difference was seen in the contamination in the HE and HI implants of the same brand (Table 1). The INP® HE system and the INP® HI system had the highest levels of contamination, with nine and seven infected units, respectively.

4. Discussions

The tiny gap (microgap) that exists between the abutment and the implant makes it easier for macromolecules and fluids from saliva and tissue fluids to infiltrate. Numerous microbes appear to be able to infiltrate along the implant's components [3].The *Streptococcus sanguis* ATCC 10556 strains was chosen for the current study because of its strong adherence to titanium and high affinity for the material. Similar to our findings, de Oliveira et al. reported that there was no correlation between *Streptococcus sanguis* ATCC10556 bacterial contamination and the size of the implant-abutment interface [8]. *Streptococcus sanguis* G9-B and *Actinomyces viscosus* T14V were tested for their adherence to dental enamel surfaces and commercially pure titanium implants by Wolinski et al. [9]; in both cases, *Streptococcus sanguis* exhibited the highest level of adherence.

Additionally, according to Edgerton et al., *Streptococcus oralis* and*sanguis*exhibited the strongest bonds when compared to the other species [10]. When comparing external-hex implants to Morse taper connection implants, Jaworski et al. found that the former were less contaminated [11]. In vitro assessment of the bacterial leakage of two distinct internal implant abutment connections is conducted by Nassar et al. They came to the conclusion that, whereas bacterial leakage appears to be unavoidable, the shape of the fixture-abutment interface greatly influences how much leakage occurs [3].Dynamic loading greatly raises the possibility of bacterial penetration at the implant-abutment junction, as Mao et al. showed [12]. Following the application of the novel sealant in both the titanium and zirconia abutments over titanium implants, Akula et al. found a considerable decrease in microbial leakage for both microorganisms [13]. Despite the various interface configurations, Faria et al. concluded that bacterial infiltration happened similarly in all three types of connections between abutments and implants [5]. According to Canullo et al., after five years of functional loads, the connections that were analysed showed pollution [6].

According to da Silva-Neto et al., the amount of 0.7 µL was greater than the implants' internal capacity, and none of the sets they examined revealed bacterial microleakage at the I-A interface [1]. In comparison to *S. Oralis, P. aeruginosa* showed a greater capacity to contaminate every link, according to Ercole et al's conclusion [14]. Nascimento et al. came to the conclusion that the implant sample samples that were recovered contained no germs prior to contamination testing (negative control). Under unloaded circumstances, bacterial species from human saliva may pass through the implant-abutment interface [15].

Table 1. Percentage of contaminated implants according to the brand name and hex type used

Implant Brand name	**Hex**		**p**
	External	**Internal**	
BIOMET3i ®	3 (20.1%)	6 (62.3%)	0.148
INP®	9 (88.0%)	7 (68.5%)	0.143
BioHorizons ®	3 (20.1%)	**0**	0.207
Adin, Israel®	**0**	1 (9.3%)	0.102
p	0.001*	0.001*	

Table 2. Values (μm) of the microspaces obtained in the interface between the abutment and implant platform

Implant Brand name	**Connection interface**					
	External- hex			**Internal- hex**		
	Average	Minimum (μm)	Maximum (μm)	Average	Minimum (μm)	Maximum (μm)
BIOMET3i ®	8.42aA	4.38	81.3	8.02aA	3.64	17.43
INP®	8.21aA	2.15	19.67	12.28aA	5.11	17.27
BioHorizons ®	5.65aA	2.68	0.00	3.18bA	1.12	10.36
Adin, Israel®	6.5aA	3.87	10.46	8.3abA	0.00	49.86

The link that has the greatest potential for bacterial microleakage is the exterior one [14]. In order to validate the findings with in vivo studies, more research is required.

5. Conclusion

The current study leads us to the conclusion that there are no statistically significant differences between the evaluated implants in terms of the amount of *Streptococcus sanguis* ATCC10556 bacteria or the size of the microspaces that are present in the implant-abutment interface.

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