Use of the electrical device on dental implant's bacterial biofilm: a preliminary in vitro study

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Abstract

Background. Mucositis and peri-implantitis are pathologies that may be encountered during dental implant rehabilitation. Therapeutic strategies for their resolution range from non-surgical to surgical treatments and aimed at eliminating the biofilm from the implant's surface, through mechanical, chemical or photodynamic agents.
Aim. The aim was to evaluate the effect of the electric field generated by the Ximplant machine on the bacterial load and on the biofilm grown on dental implants.
Materials and Methods. Ten dental implants were brought into contact with a donor's saliva, then five implants were treated with the electric field and four were not treated.
Bacterial biofilm was then measured by resazurin assay, both on treated and untreated implants.
Results. The study showed the preliminary success of the electrofield in reducing the microbial population and destroying the clinical biofilm, compared with a sterile implant as control.

Key words: mucositis, periimplantitis, biofilm removal.

Introduction

The treatment of choice for the resolution of both partial and total edentulism of patients consists in the use of dental implants (1-5). In recent decades, their use has exponentially evolved. Mucositis and peri-implantitis are pathologies that may be encountered during dental implant rehabilitation. (6-10).
The first one is characterized by all the signs of inflammation without radiographic signs of bone loss, while the second one is also characterized by purulent exudate and radiographic signs of peri-implant bone loss.
When we talk about peri-implant bone loss we must differentiate it from biological factors, such as physiological remodeling or mechanical stress. The inflammatory-bacterial etiology determines resorption between the interface of the bone and implant and its consequent loss (11-16).
In the literature, therapeutic strategies for the resolution of these two pathological conditions range from non-surgical to surgical treatment and aimed at eliminating the superficial biofilm, through mechanical and/or chemical agents (7).
As demonstrated in many scientific papers, these techniques present critical issues linked to the partial elimination of bacteria and surface contaminants.
In recent years the metallurgical industry has conducted studies on the electrochemical

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cleaning of metal surfaces to eliminate and decontaminate biofilm and prevent its formation.
The electric current would act on the electrochemical bonds of the polysaccharide particles of the biofilm layer, determining a reduction in hydrogen bonding, also breaking the bonds that determine adhesion to surfaces. (17)
This new technique would allow the elimination and decontamination of the metal implant surfaces while keeping them intact, which does not happen with mechanical procedures that alter their shape (10).
The aim of the work was to evaluate the effect of the electric field on the reduction and decontamination of bacterial plaque on the implant surface.

MATERIALS AND METHODS

Bacterial contamination of dental implants

Ten dental implants in grade 4 titanium (sandblasted and etched with double acid attack) with a length of 13 and a diameter of 4,2 (SEVENTIN-ONE by company Maco dental care Salerno Buccino) were used.
A healthy volunteer who presented with active peri-implant pathology, with signs of inflammation, suppuration and with radiographic signs of peri-implant bone loss was recruited to donate his saliva.
The present study has been conducted in accordance the principles and guidelines of the Declaration of Helsinki. The informed consent was obtained from all individual participants included in the study.
The salivary collection was performed in the morning and the subject was asked not to practice the oral hygiene routine before the collection 5 ml of saliva was collected.
Contamination of the implants was performed using a bacterial culture in the logarithmic phase of growth, prepared by growing ten colonies of saliva in 5 ml of Brain Hearth Infusion (BHI) broth (Oxoid ThermoFisher Scientific, US) supplemented with 5% defibrinated sheep blood in an anaerobic environment for 96 hours at 37°C.
The bacterial suspension was adjusted to the OD of 0.5 McFarland scale and subsequently diluted 1:1000. The formation of bacterial biofilm on the implants occurred by incubating the devices in a sterile vial (Eppendorf Safe-Lock Tubes, Eppendorf Italy) with 900 µL of bacterial suspension prepared as described above. The samples were incubated in an upright position for 48 hours at 37°C. After incubation, the implants were washed three times in a sterile 0.9% NaCl solution to remove the planktonic form of non-adherent bacteria.

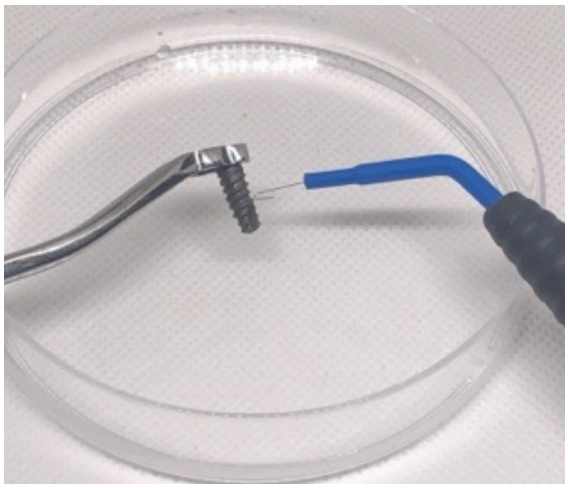

Figure 1. Treatment chamber

Figure 2. Device

Electrical treatment of implants

After bacterial contamination, five implants were transferred to the treatment chamber with the addition of 100 µl of 0.9% NaCl solution and treated using the "Peri-implantitis Protocol" of the X-IMPLANT instrument. (Figure 1, 2)
This protocol consisted of four cycles of electrical current (alternating electrical current at 625 kHz, 260 Vpp, 15 W and 180 mA) performed on the implant according to the programmed times of the machine, the electrode was positioned in 4 tangential positions peripherally at 90 ° from the previous position. Once the treatment phase with the Ximplant instrument was completed, the implants were further washed with 0.9% NaCl solution.
Four implants were not treated. One implant was sterile and incubated with 900 µl of BHI was used as a negative control.
Both treated and untreated implants were added to a reagent, resazurin (Labbox italia srl) and incubated for visual evaluation at 2 hours, one day, two days and the final third day of the experimental procedures. (19) Table 1.

Statistical analysis

Descriptive statistics was performed. Chi-squared test was used to assess significant differences between the two groups (treated vs not treated) in each reference time with $p<0.05$. (Figure 1 and Table 2)

Results

All treated implants did not show any color change, as the control sterile implant.
All not-treated implants showed a color change already after two hours.

Discussion

It is now proven that the presence of bacteria leads to the formation of biofilm on all surfaces, both biological and non-biological, as demonstrated by scientific studies on biofilm, the first to be formed is made up of beneficial bacteria called commensals. (12-15)
However, the reduced host response and environmental modifications caused by clinical alterations can lead to a

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Table 1. Row data. Negative = no color change; Positive= color change

Implants #	After 2 hrs	After 1 day	After 2 days	After 3 days
C 1	Negative	Negative	Negative	Negative
2	Negative	Negative	Negative	Negative
3	Negative	Negative	Negative	Negative
4	Negative	Negative	Negative	Negative
5	Negative	Negative	Negative	Negative
6	Negative	Negative	Negative	Negative
7	Positive	Positive	Positive	Positive
8	Positive	Positive	Positive	Positive
9	Positive	Positive	Positive	Positive
10	Positive	Positive	Positive	Positive

Table 2. Chi-square analysis. dF= degree of freedom.

Time	χ^2 value	dF	P value
T1 =after 2 hrs	9000	1	0.003
T2 = after 1 day	9000	1	0.003
T3= after 2 days	9000	1	0.003
T4= after 3 days	9000	1	0.003

shift in the commensal microbial flora towards the development of pathogenic species, an event called dysbiosis. Dysbiosis causes an increase in the production of inflammatory mediators, which induce the production of toxic products in the host cell which in turn lead to the destruction of the tissues around the implant.
In the literature, various surgical and non-surgical strategies have been introduced for the elimination of pathological biofilm from surfaces. (20-24)
Both are based on periodontal treatments and prevention because it is considered essential to give appropriate hygiene instructions to the patient to reduce the bacterial load keeping the peri-implant tissues healthy (25-27).
In some scientific works where the use of oral antiseptics such as 12% chlorhexidine after appropriate mechanical debridement was used, it did not improve the scores of gingival bleeding after probing (BOP) compared to control groups where mechanical debridement alone was used.
Even the potential beneficial effects (reduction of BOP and deep bleeding) hypothesized using systemic antibiotics (azithromycin) failed three/six months after treatment, just as the use of probiotics had no benefit compared to mechanical therapy. (28, 29)
On the other hand, the use as an alternative to mechanical therapy such as, for example, the use of ultrasound instruments, glycine sandblasting sprays or YAG lasers has a good result in clinical terms with reduction of BOP, compared to mechanical debridement alone.

	T1=after 2hr		
PP	Negative	Positive	Total
No	0	4	4
Yes	5	0	5
Total	5	4	9

	T2=after 1day		
PP	Negative	Positive	Total
No	0	4	4
Yes	5	0	5
Total	5	4	9

	T3=after 2 days		
PP	Negative	Positive	Total
No	0	4	4
Yes	5	0	5
Total	5	4	9

	T4 after 3 days		
PP	Negative	Positive	Total
No	0	4	4
Yes	5	0	5
Total	5	4	9

Contingency tables. PP= peri-implantitis protocol yes/no.

The use of both photonic and laser techniques, however, mostly control the progression of the peri-implant pathology rather than resolve it (30,31).
The poor results of bacterial decontamination regarding these techniques could be attributed to the difference in the titanium surface compared to that of the dental root. This implies that the re-osseointegration phase is deficient with the interposition of fibrous tissue between the bone and the implant as demonstrated by histological studies. (32, 33).
However, in recent years, electrochemical treatments for the decontamination of biofilm have appeared. They cause a polarization of metal surfaces, preventing microorganisms from attaching and breaking the anchoring bonds to the structures. Furthermore, the electrochemical activity determines a change in PH with the formation of oxidizing ions which reduce the number or kill the bacteria present. (34)
Lately, some scientific works have dealt with implant decontamination from biofilm using low intensity direct currents. They have given good results as no live bacteria were present at the anode level, while at the cathode the colonies were three times reduced. (35)
Since Our study aimed to evaluate the alternating current on a contaminated implant surface, the results allow us to evaluate the good response of bacterial decontamination.
The data from this work should be verified with other in vitro and in vivo works due to the characteristic of the oral bacterial flora which varies from above to below the gums, with a larger sample size.

Conclusions

Considering the limits of this scientific work, the results push us to continue and follow the path of using electric current in peri-implant treatment therapy.
Expanding new treatment strategies.

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