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RESEARCH ARTICLE

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-implant disease are pathological conditions found following bacterial colonization on the peri-implant soft tissues and on the implant fixture during implant-prosthetic rehabilitation, from single edentulism to full-arch rehabilitation.
The therapeutic approaches to the two pathological conditions use surgical and non-surgical therapeutic protocols, with the aim of eliminating the bacterial biofilm from the implant surface, through the use of mechanical, chemical or photodynamic agents.
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 the dental implants.
Materials and methods. Twenty-eight dental implants were contaminated with the saliva of a donor, and subsequently fifteen implants were treated with the electric field generating machine while twelve were not treated. The bacterial biofilm was then measured by resazurin assay, both on treated and untreated implants.
Results. The results revealed a difference between treated and untreated implants in terms of biofilm activity,as assessed by color change using the resazurin assay. Treated implants (n = 15) showed no color change across all observation time points (2 hours, 1 day, 2 days, 3 days), indicating an absence of bacterial activity or residual biofilm. Conversely, all untreated implants (n = 12) exhibited a consistentcolor change starting at 2 hours, suggesting persistent biofilm activity. A sterile implant used as a negative control (n = 1) showed no color change throughout the experiment, confirming the absenceof contamination
Conclusion. The study showed preliminary success of the electrofield in reducing microbial populations and destroying clinical biofilm, compared to a sterile implant as a control.

Keywords: Mucositis, peri-implantitis, bacterial load, dental implant

INTRODUCTION

Implant-prosthetic rehabilitation is the treatment of choice for functional and aesthetic restoration, both in cases of single edentulism and in cases of total arch rehabilitation [1-5].

Mucositis and peri-implant disease are pathologies that can be encountered during dental implant rehabilitation [6-10]. Mucositis is characterized by the distinctive signs of inflammation with the absence of radiographically visible loss of supporting alveolar bone. Peri-implant disease is also characterized by purulent exudate and radiographic signs of peri-implant bone loss. Peri-implant bone loss can have a bacterial etiology, determined by the action of the peri-implant bacterial biofilm, as well as by intraoperative and prosthetic surgical causes. This type of pathological bone resorption must be differentiated from biological factors, such as physiological remodeling or mechanical stress. The inflammatory-bacterial etiology determines the resorption between the bone-implant interface and its consequent loss [11-16].

In literature, the therapeutic strategies for the resolution of these two pathological conditions range from non-surgical to surgical treatment and aim at the elimination of the surface biofilm, through mechanical and/or chemical agents[7]. As demonstrated in many scientific works, these techniques present critical issues related to the partial elimination of bacteria and surface contaminants [46-52]. In recent years, the metallurgical industry has conducted studies on the electrochemical cleaning of metal surfaces to eliminate and decontaminate the biofilm and prevent its formation by acting on the electrochemical bonds of the biofilm layer, also breaking the bonds that determine adhesion to the surfaces[17]. This new technique would allow the decontamination of the surfaces of dental implants while maintaining their shape intact, unlike current mechanical procedures [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 surface of the implant.

Materials and methods

The study protocol applied, regarding both the bacterial contamination of the fixtures and their electrical treatment, follows Falisi et al. [54].

Bacterial contamination of dental implants.

Twenty-eight grade 4 titanium dental implants (sandblasted and double acid etched) with a length of 13 and a diameter of 4.0 (In-Kone® by Global D, France) were used.
This study was conducted in accordance with the principles and guidelines of the Declaration of Helsinki. Five ml of saliva was collected from the volunteer patient with active peri-implant disease, with signs of inflammation, suppuration and radiographic signs of peri-implant bone loss. On the morning of collection, he was asked not to practice his usual home oral hygiene routine. Implant contamination was performed using a bacterial culture in the logarithmic phase of growth, prepared by culturing ten colonies of saliva in 5 ml of Brain Hearth Infusion (BHI) broth (Oxoid ThermoFisher Scientific, USA) 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. Bacterial biofilm formation on the implants occurred by incubating the devices in a sterile vial (Eppendorf Safe-Lock Tubes, Eppendorf Italia) with 900 µL of bacterial suspension prepared as described above. The samples were incubated in a vertical position for 48 h 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.

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 (LED S.P.A., Aprilia, Italy) (fig 1, 2).

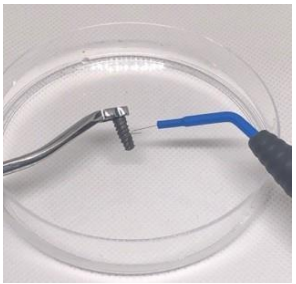

Fig.1 Treatment chamber **Fig.2** Device

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 previousposition. 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 theexperimental procedures[19] (Table 1).

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	Negative	Negative	Negative	Negative
8	Negative	Negative	Negative	Negative
9	Negative	Negative	Negative	Negative
10	Negative	Negative	Negative	Negative
11	Negative	Negative	Negative	Negative
12	Negative	Negative	Negative	Negative
13	Negative	Negative	Negative	Negative
14	Negative	Negative	Negative	Negative
15	Negative	Negative	Negative	Negative
16	Negative	Negative	Negative	Negative
17	Positive	Positive	Positive	Positive
18	Positive	Positive	Positive	Positive
19	Positive	Positive	Positive	Positive
20	Positive	Positive	Positive	Positive
21	Positive	Positive	Positive	Positive
22	Positive	Positive	Positive	Positive
23	Positive	Positive	Positive	Positive
24	Positive	Positive	Positive	Positive
25	Positive	Positive	Positive	Positive
26	Positive	Positive	Positive	Positive
27	Positive	Positive	Positive	Positive
28	Positive	Positive	Positive	Positive

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 $p<0.05$.

Results

The results revealed a difference between treated and untreated implants in terms of biofilm activity, as assessed by color change using the resazurin assay. Treated implants (n = 5) showed no color change across all observation time points (2 hours, 1 day, 2 days, 3 days), indicating an absence of bacterial activity or residual biofilm. Conversely, all untreated implants (n = 4) exhibited a consistent color change starting at 2 hours, suggesting persistent biofilm activity. A sterile implant used as a negative control (n = 1) showed no color change throughout the experiment, confirming the absenceof contamination (fig. 2).

	T1=after 2hr		
PP	Negative	Positive	Total
No	0	12	12
Yes	15	0	15
Total	15	12	27

	T2=after 1day		
PP	Negative	Positive	Total
No	0	12	12
Yes	15	0	15
Total	15	12	27

	T3=after 2 days		
PP	Negative	Positive	Total
No	0	12	12
Yes	15	0	15
Total	15	12	27

	T4 after 3 days		
PP	Negative	Positive	Total
No	0	12	12
Yes	15	0	15
Total	15	12	27

Fig. 2. Contingency tables. PP= peri-implantitis protocol yes/no.

The χ^2test demonstrated significant differences between treated and untreated groups at all time points, with $\chi^2 = 9.000$, $p = 0.003$. These findings confirm the effectiveness of the electric field treatment in significantly reducing bacterial activity. (Table 2).

Table2.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

The absence of biofilm activity in treated implants contrasts sharply with the consistent biofilm activity in untreated ones.

Table 1 and Figure 1 summarize these findings, illustrating the significant difference in bacterial activity between the two groups. In conclusion, all treated implants did not show any color change, as the control sterile implant and all not-treated implants showed a color change already after two hours.

As demonstrated by scientific studies on biofilm, the presence of bacteria leads to the formation of biofilm on all surfaces, both biological and non-biological [12-15]. The first layer of biofilm to form is made up of beneficial bacteria called commensals. Due to changes in the intraoral habitat and the reduced host response, a state of dysbiosis is triggered that determines a shift of the commensal microbial flora towards the development of pathogenic species. Dysbiosis causes an increase in the production of inflammatory mediators, which induces the production of toxic products in the host cell that in turn lead to the destruction of the tissues around the implant [43-46]. In the literature, several 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 the patient the appropriate hygiene instructions to reduce the bacterial load while maintaining healthy peri-implant tissues [25-27].

In some scientific works in which the use of oral antiseptics such as 12% chlorhexidine was used after adequate mechanical debridement, it did not improve the scores of gingival bleeding after probing (BOP) compared to the control groups in which only mechanical debridement was used.

Even the potential beneficial effects (reduction of BOP and deep bleeding) hypothesized using systemic antibiotics (azithromycin) failed three/six months after treatment, as well 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 the use of ultrasonic instruments, glycine spray sandblasting or YAG laser has a good clinical outcome with BOP reduction, compared to mechanical debridement alone [40,43]. The use of both photonic and laser techniques, however, mostly control the progression of peri-implant pathology rather than resolve it [30,31].

The poor results of bacterial decontamination compared to 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]. A strategy that seems to show advantages in the prevention and treatment of peri-implantitis is the ultraviolet (UV) photofunctionalization of the implant fixture surface. UV irradiation of surfaces has shown an antimicrobial effect, as it converts the hydrophilicity of the implant surface, increasing attachment, retention, proliferation and osteogenic cells. However, in recent years, electrochemical treatments for biofilm decontamination have appeared. They cause a polarization of the metal surfaces, preventing microorganisms from attaching and breaking the anchoring bonds to the structures. In addition, the electrochemical activity determines a change in pH with the formation of oxidizing ions that reduce the number or kill the bacteria present [40.53.]

Recently, some scientific works have dealt with the decontamination of plants from biofilm using low intensity direct currents. They have given good results since there were no live bacteria at the anode, while at the cathode the colonies were reduced threefold [40,41,43].

Since our study aimed to evaluate the alternating current on a contaminated plant surface, the results allow us to evaluate the good response of bacterial decontamination.

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.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical approval

The study was approved by the University ethics committee and was conducted in accordance with the Declaration of the World Medical Association.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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