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Use of electrical field for biofilm implant removal

S. BERNARDI[1], E. QORRI[2], G. BOTTICELLI[1], A. SCARANO[3], G. MARZO[1], R. GATTO[1], A. GRECO LUCCHINA[4], C. MORTELLARO[4], E. LUPI[5], C. RASTELLI[1], G. FALISI[1]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila, L' Aquila, Italy
[2]Faculty of Medical Sciences, Albanian University, Tirane, Albania
[3]Department of Innovative Technology in Medicine and Dentistry, University of Chieti-Pescara, Chieti, Italy
[4]Stomatological Diseases and Oral Surgery, Saint Camillus International University, Rome, Italy
[5]San Salvatore Hospital, L'Aquila, Italy

S. Bernardi, G. Botticelli, A. Scarano and G. Falisi contributed equally to this work

Abstract. – OBJECTIVE: New methods for biofilm removal are being investigated. A recent new one involves the use of the electric field for biofilm removal. In particular, electrolytic cleaning works on the adhesion forces of the biofilm on the surfaces, with few studies showing promising results in decontamination and implant re-integration in the bone. This study aims at assessing the effect of a new decontamination device that implies the electric field for implant-biofilm removal.

MATERIALS AND METHODS: Three implants affected by peri-implantitis were selected for the study. After the treatment, the implants were observed by the Scanning Electron Microscopy.

RESULTS: All three samples showed no microbial biofilm in the application area, while the rest of the surface observed was covered with microbial biofilm, with an intensely thickened bacterial population.

CONCLUSIONS: Peri-mucositis and peri-implantitis prevention and early treatments are essential for implant maintenance, thus saving the surrounding hard and soft tissues. The technological innovation is providing electrolytic devices which act not only on the microbial population but on the biofilm adhesion to the implant surface, with promising results for a new and valid therapeutic option.

Key Words:
Peri-Implantitis, Peri-Mucositis, Electrolytic Cleaning.

Introduction

Implant dentistry is an established branch of dentistry, including the rehabilitation of partial or total edentulism by using titanium alloy-fixtures[1-5]. Unfortunately, what researchers and clinicians thought to be a long-term therapeutic option, especially in those cases of dental loss due to periodontitis, has become the source of a new inflammatory disease: peri-implantitis[6,7]. According to the 2017 scheme American Academy of Periodontology and the European Federation of Periodontology, the healthy status of peri-implant tissues is defined as "an absence of visual signs of inflammation and bleeding on probing"[8]. When inflammatory diseases around implants are present, two conditions are identified and classified: peri-implant mucositis and peri-implantitis. The former presents bleeding on probing, with inflammatory characteristics and reversibility, and it is characterized to be plaque-dependent. Beyond being plaque-dependent, the latter shows the loss of surrounding bone tissue and inflammation of the peri-implant mucosa[6]. The oral environment is characterized by the presence of a biofilm covering all the surfaces: dental, gingival, mucosal and also the implant ones. This condition leads to the formation of micro-environments where the biofilm characteristics differ in composition. Therefore, the biofilm covering implant surfaces owns peculiar characteristics and microbial composition. Several studies[9-11] have reported that the peri-implantitis biofilm microbial population mainly comprises *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia,* and *Treponema denticola*. This peculiar biofilm can trigger an inflammatory

Corresponding Author: G. Botticelli, DDS; e-mail: botticelligl@gmail.com

process, with soft-tissue suffering, the formation of a pocket around the implant and, finally, the resorption of peri-implant bones. Since peri-implantitis can be due to metal particles released in the bone tissue[12] and/or due to this harmful biofilm, different treatment options have been investigated and introduced to decrease the microbial load and the biofilm presence. These treatments include mechanical instrumentation, as well as manual debridement, ultrasonic debridement, air-abrasive device and laser decontamination[13,14]. Mechanical treatment limitations are particularly critical when the rough portion of the implant surface is involved[15,16]. Hence, combining the mechanical and local application of antibiotics and/or antiseptics is one of the most promising strategies to address peri-implantitis[17]. However, administering antiseptics or antibiotic molecules can lead to the development of antibiotic resistance, or even antibiotics are not always possible to administer. New methods for biofilm removal are being investigated. A recent new one[18] includes the use of the electric field for biofilm removal. In particular, electrolytic cleaning works on the adhesion forces of the biofilm on the surfaces, and few studies[18,19] have been showing promising results in decontamination and implant re-integration in the bone. The current study aims at assessing the effect of a new decontamination device that uses an electric field for implant-biofilm removal.

Materials and Methods

Samples and Study Design

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. Three implants affected by peri-implantits were selected for the study. In particular, these samples (named in the text as implantX, implantX36 and implantX46) had an open defect according to the classification of L. Vanden Bogaerde[20]. The samples presented a biofilm of unknown microbiological composition adhered to the implant walls. The implants were not mobile, but had more than 80% bone loss, purulent exudate and positive bleeding on probig, with a pocket depth > 6 mm. More in details, implantX36 and implantX46 were extracted from a 67-year-old female patient, approximately 11 years after prosthetic loading and in the context of a compromised peri-implant situation (Figure 1). The implantX was extracted from a 71-year-old male patient after about 11 years in the dental arch. The radiographic status showed another compromised situation in this case, indicating the need of implant removal (Figure 2). The XIMPLANT machine (LED S.P.A., Aprilia, Italy) consists of a unipolar electrode and a ‘bunch’ electrode, (i.e., an electroconductive stick) that in clinical practice is placed in the patient’s hand to close the circuit, to allow te passage of electric current. The “PERIMPLANTITIS” (in original on manufacturer instructior language “PERIMPLANTITE”) protocol includes, for each treatment, 4 cycles lasting 3 seconds each and interspersed with a 2-second pause. The alternating current (AC) flows through the unipolar electrode with

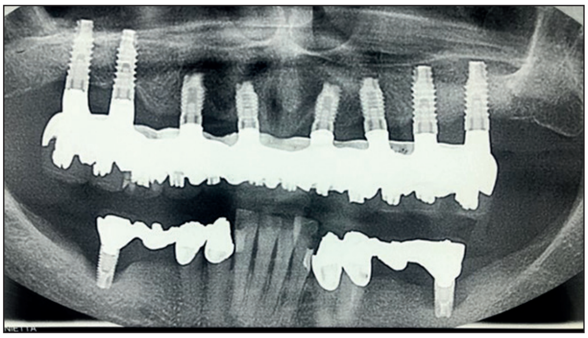

Figure 1. Patient Orthopantomogram. The implantsX36 and X46, present severe peri-implant compromission; generalised bone resorption is present in all implant elements, albeit to varying degrees.

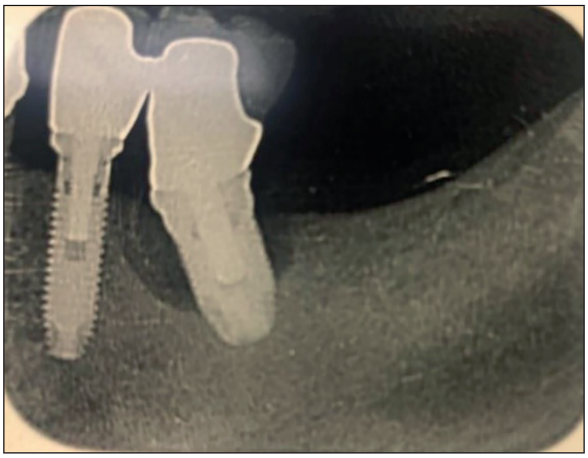

Figure 2. ImplantX endo-oral radiograph. Low level of crestal bone available.

a sine wave at 625 kHz, 260 Vpp peak-to-peak voltage and 15 W power, with an amperage of 180 mA, (manufacture''s declared data). The samples are kept in physiological saline solution and maintained at -20°C. Successively, they are warmed to a temperature of 37°C and treated with the PERIMPLANTITIS protocol of the XIMPLANT machine. Then, the surfaces are observed using the Scanning Electron Microscopy (SEM – GEMINISEM 500, Carl Zeiss Microscopy GmbH, Oberkochen, Germany). The spherical diamond tip (Figure 3) is used on the vestibular surface of the implant neck to make the treated area evident.

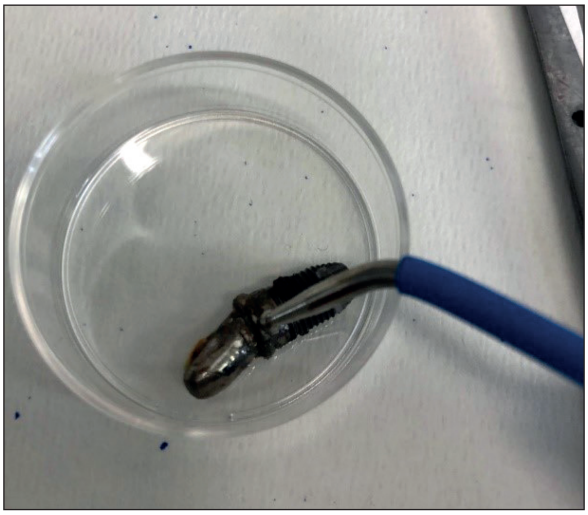

Figure 3. The spherical tip used for the experiment, with sample X in the background.

SEM Protocol

The samples are then fixed in 2.5% glutaraldehyde for 4 h, immersed in PBS for 2 h and dehydrated on an ascending alcohol scale (50% for 20 minutes, 75% for 20 minutes, 80% for 20 minutes, 95% for 20 minutes twice, and 100% for 20 minutes twice), and allowed to dry at room temperature for 48 h. They are then placed on the holders for observation under the SEM, using the BSD4 probe. Once the application spot is identified using low magnifications, several spots are observed at different magnifications (500×, 1,000× and 2,000×).

Results

All three samples showed an absence of microbial biofilm in the application area, while the rest of the surface observed was covered in microbial biofilm, with an intensely thickened bacterial population (Figure 4). The implantX sample presented the area where the handpiece was applied (Figure 5) at 500×, 1,000x and 2,000×. The darker areas represented the presence of organic material of a microbial nature, then confirmed at higher magnifications, showing that in the area where the tip was not applied organic material remained adhered to the surface. Concerning the implantX36 sample, the investigated surfaces appeared covered with organic material on the spires and neck even at low magnification (Figure 4B). At higher magnifications, it was interesting to note that the application area was free from biofilm, while the interface with the organic material was typically composed of biofilm and microbial material (Figures 6 and 7). The sample implantX46 was covered with organic material on the coils even at low magnifications (Figure 4C). High magnifications confirmed that there was a bacterial population at the interface in the untreated area, while in the treated area the implant surface was free from microbial biofilm (Figure 8).

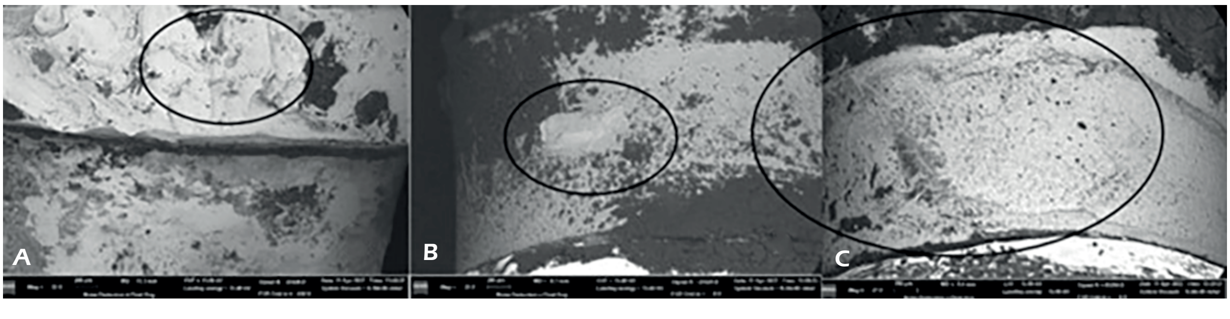

Figure 4. SEM microphotographs of the three samples at 32× magnification **A**, sample implant; (**B**), sample implantX36; (**C**), sample implantX46. In all of the three pictures, the circled area indicates the point of application of the tip.

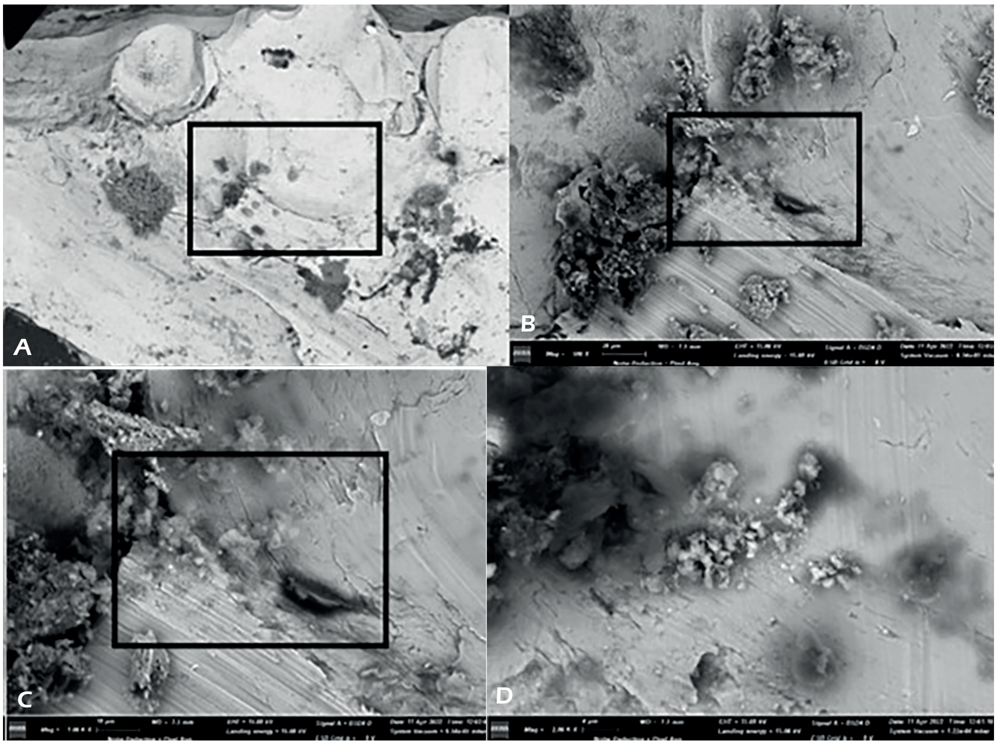

Figure 5. Sample implantX. Magnifications a t 5 00× (**A**), 1,000× (**B**) and 2,000× (**C-D**) of the application area At 2,000×, the rounded microbial population can be appreciated.

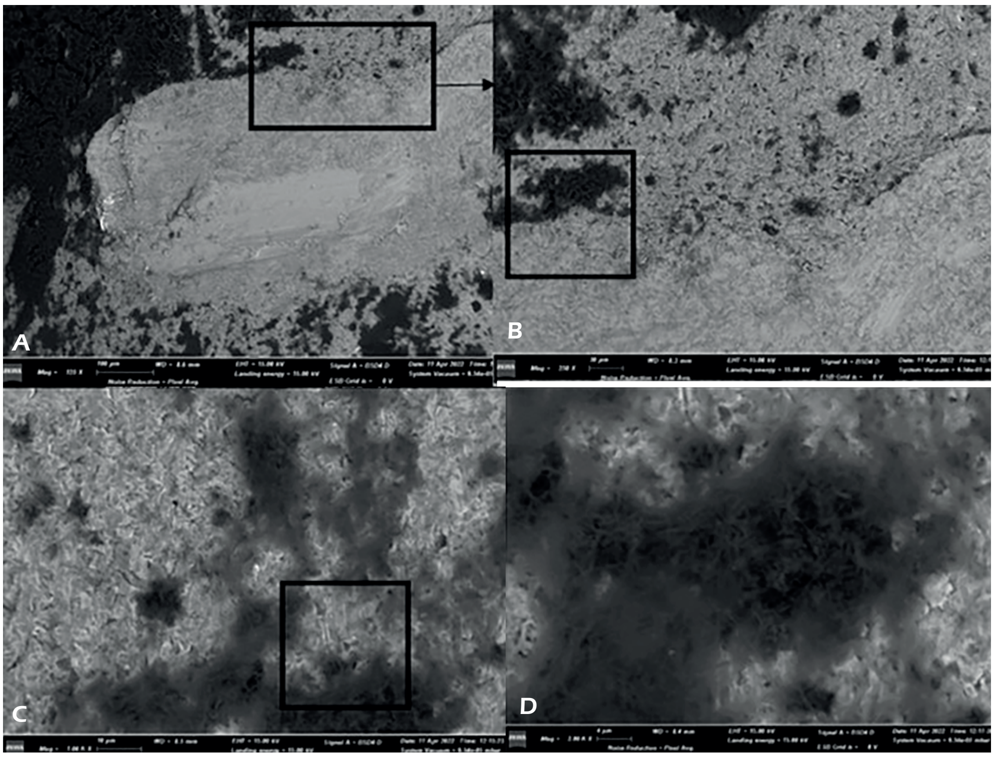

Figure 6. Sample implantX36. Magnification 125× of the treated area (**A**) and inset magnifications at 250× (**B**), 1,000x (**C**) and 2,000× (**D**) of the interface where the microbial biofilm is present. The bacterial presence is characterized by rounded and fusiform elements intensely reticulated.

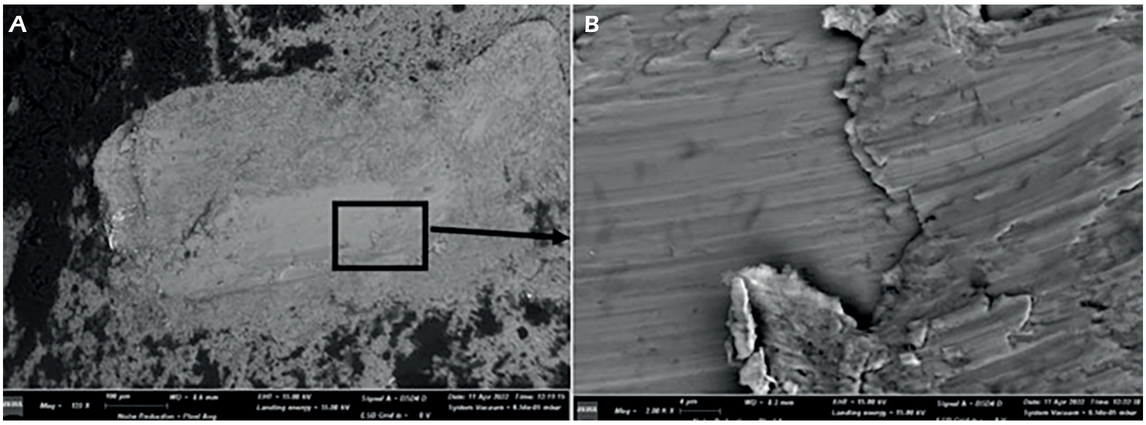

Figure 7. Sample implantX36. Magnification 125x (**A**) of treated area and inset magnification 2,000x (**B**) of treated area Biofilm and microbial population absent.

Discussion

The biofilm covering the surfaces of biomedical devices has always represented a huge challenge, especially in the hospital environment, since it represents a source of potential and life threating infections[16,17]. Therefore, several options[21-25] have been studied and introduced to prevent and remove the microbial biofilm. In case of implant dentistry, the preservation of the fixture is important both for the rehabilitation of the edentulism and to avoid the implant to become a source of a new infection[21]. Therefore, the maintenance of the fixture begins from the preventive measures, such as patient motivation, as well as a correct prosthetic project. Then, oral

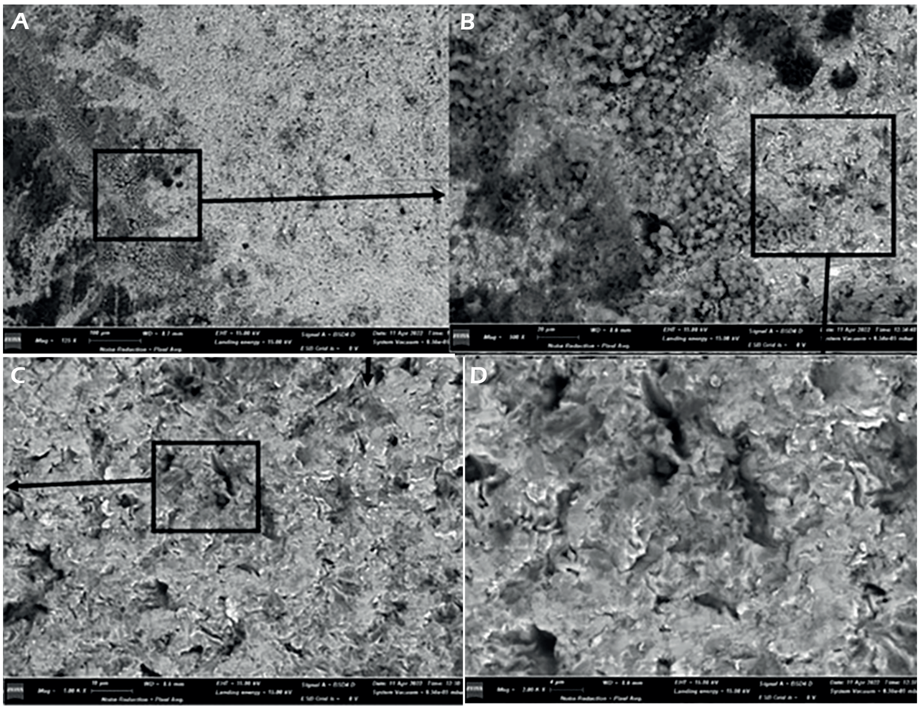

Figure 8. Sample implantX46. Different magnifications (**A**, 125x; **B**, 250x; **C**, 1,000x; **D**, 2,000x) of the interface. On the untreated side, biofilm with a rounded microbial population (presumably *Streptococcus sp*) can be significantly observed, while the treated side is represented by the decontaminated implant surface..

professional hygiene sessions, prescription of the correct mouthwashes and the teaching of correct use of the home dental hygiene tools represent primary intervention strategies to keep low the microbial load[22]. The current study showed a different and relatively new method for the biofilm removal; indeed, the life of microbial community living in biofilm is also influenced by the molecules released by those microorganisms destroyed by microbicidal substances. The releases of extracellular polymeric substance (EPS), for example, can affect the genetic expression of resistance protein[23]. The removal and complete disruption of the biofilm from the surface is the primary aim of peri-implantits treatment, whether accompanied by a non-surgical or surgical approach. As reported by Marín-Jaramillo et al[24], the frequency of the sessions for implant maintenance should depend on the risk profile of the patient. The regular adherence to supportive periodontal therapy is recommended as the best way to prevent the peri-mucositis and peri-implatitis occurrence, to remove biofilm in the initial and reversible stages[25]. The supportive and preventive therapies include the education of the patient to the oral hygiene, accurate inspection, the use of mechanical therapy (manual and ultrasonic debridment, air polishing) and eventual use of antiseptics and antibiotics. XIMPLANT machine, working on the adhesion and static Van der waalls strengths can be a useful tool in the prevention and supportive therapy. The treatment of the peri-mucositis and peri-implantitis includes non-surgical and surgical procedures. In case of peri-mucositis, usually the non-surgical procedure (manual and ultrasonic debridment, air polishing, use of clorexidine and of local antibiotics), eventual use of antiseptics and antibiotics and the strictly adherence of the patient to a maintenance and regular program are effective in the regression and in the prevention of the peri-implantitis[22]. The protocols for prevention and treatment of perimucositis are the first attempt to treat periimplantitis; however, Roccuzzo et al[26] report that the non-surgical procedure should be used to prepare the peri-implant tissue to the surgical therapy.

Surgical procedure for treating peri-implantitis include two different approaches: resective approach and regenerative approach. The former includes the removal of the inflammatory tissue and the bone recontouring with the use of antimicrobial molecules and implantoplasty; the latter includes the use of biomaterials, such as demineralized and deproteinized bovine bone[27,28] or Platelet Rich Fibrin[29] as scaffold to compensate the peri-implant defect left after the debridment. A preventive and an interceptive approach to the peri-implant disease allows to maintain the implant and spare the surrounding bone tissue. The use of electrolysis for biofilm removal has been considered[18,19] in the last years as an alternative method for maintenance and debridement in case of treatment of peri-implantitis (also in cases of surgical therapy). Ratka et al[18] experimented *in vitro* the use of electrolysis for implant surfaces decontamination, simulating clinical oral conditions. The study compared the electrolysis *vs.* the air-polishing, with results statistically significant in favor of the electrolysis. These *in vitro* results have been lately confirmed by an *in vivo* study by Bosshardt et al[29], who assessed in their case-series the reosseointegration of implants affected by severe peri-implantis after electrolytic exposure and regenerative procedure. In these studies, the tested electric device acted not directly on the biofilm, but using the activation of a fluid solution, which broke the bonds between the biofilm and the implant surface and acted as microbicidal agent. In our study, even though the morphological observation confirmed the removal of the biofilm in the area of application, the device protocol did not include the use of any fluid solution, acting directly and only on the biofilm adhesion. The area of application resulted clean and free from microorganisms traces, which were present instead at the interfaces and along the not treated areas.

Limitations

The small size of the sample has limited the significance of the present study; however, the promising results, together with the data available in literature, open a new window on the therapeutic options for peri-implantisis prevention and treatment. More *in vitro* studies corroborated by *in vivo* model trials are necessary to confirm the efficacy of the electrolysis in biofilm removal.

Conclusions

Peri-mucositis and peri-implantitis prevention and early treatments are essential for implant maintenance, saving the surrounding hard and soft tissue. The technological innovation is providing electrolytic devices which act not only on the microbial population, but also on the biofilm adhesion to the implant surface, with promising results for a new and valid therapeutic option.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

SB, GB and GF designed the research study. SB and EL performed the research. CR analyzed the data. EQ and SB wrote the manuscript. AS, GM, AGL, RG and GF revised the final version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval

The nature of the study is experimental *in vitro*, therefore the ethics approval was waived.

Informed Consent

Patients gave their consent to donate the implants to research purposes. The informed consent was obtained from all individual participants included in the study.

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ORCID ID

Sara Bernardi: 0000-0001-6130-8533; Erda Qorri: 0000-0002-5079-6815; Gianluca Botticelli: 0000-0002-0493-2468; Antonio Scarano: 0000-0003-1374-6146; Giuseppe Marzo: 0000-0001-9088-7749; Roberto Gatto: 0000-0002-1965-6080; Alberta Greco Lucchina: 0000-0002-1985-4263; Carmen Mortellaro: 0000-0002-1150-1906; Ettore Lupi: 0000-0001-6993-9911; Giovanni Falisi: 0000-0001-5166-9861.

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