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Rough Dental Implant Surfaces and Peri-Implantitis: Role of Phase-Contrast Microscopy, Laser Protocols, and Modified Home Oral Hygiene in Maintenance. A 10-Year Retrospective Study

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Abstract: The aim of this study was to evaluate two different kinds of rough implant surface and to assess their tendency to peri-implantitis disease, with a follow-up of more than 10 years. Data were obtained from a cluster of 500 implants with Ti-Unite surface and 1000 implants with Ossean surface, with a minimum follow-up of 10 years. Implants had been inserted both in pristine bone and regenerated bone. We registered incidence of peri-implantitis and other causes of implant loss. All patients agreed with the following maintenance protocol: sonic brush with vertical movement (Broxo), interdental brushes, and oral irrigators (Broxo) at least two times every day. For all patients with implants, we evaluated subgingival plaque samples by phase-contrast microscopy every 4 months for a period of more than 10-years. Ti-Unite surface implants underwent peri-implantitis in 1.6% of the total number of implants inserted and Ossean surface implants showed peri-implantitis in 1.5% of the total number of implants. The total percentage of implant lost was 4% for Ti-Unite surfaces and 3.6% for Ossean surfaces. Strict control of implants leads to low percentage of peri-implantitis even for rough surfaces dental implants.

Keywords: bacteria; dental implants; hydrogen peroxide; laser; peri-implantitis; photodynamic therapy; preventive dentistry

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1. Introduction

Dental implants are widely used in dentistry, with a continuous increase in the number of patients treated and a parallel continuous developing of new techniques and technologies available for clinicians.

A study by Elani et al. estimated that there will be a prevalence of dental implants from 5.7% to 23% in the American population in 2026, so it is clear how important this treatment option is [1].

Despite this large distribution of dental implants, there are still some problems that can occur during treatment—lack of osseointegration, peri-implantitis, occlusal overloading, fixture fractures, and abutment screw loosening [2,3].

In particular, peri-implantitis is surely one of the major problems that can occur.

In 2017, a new classification was proposed by the American Academy of Periodontology and the European Federation of Periodontology—a presence of bleeding or suppuration with gentle pressure on probing and some variations from previous examinations, such as increased probing depth and increased bone loss. In the case of absence of previous examinations, peri-implantitis is defined as presence of bleeding and/or suppuration on

gentle probing, probing depths $\geq$ 6 mm, and bone levels $\geq$ 3 mm apical to the most coronal portion of the intraosseous part of the implant [4].

Moreover, it is very important to consider that different classifications, with restrictive diagnostic criteria, are associated with significantly reduced presence of peri-implantitis [5].

In the etiology of peri-implantitis, in order of severity, we can consider:

1. Microbial contamination (periodontal disease) [6];
2. Bone quality (>PI in regenerated bone: caution is required in the event of poorly vascularised bone) [7];
3. Surgical damage. Thermal injury to bone [8];
4. Soft Tissues morphology (i.e., the absence of keratinized mucosa around dental endosseous implants has been associated with a higher susceptibility to plaque-induced tissue destruction [9], but still there is no consensus on this argument [6];
5. Implant surface [10];
6. Abutment connection [11];
7. Prosthetic damage (design, overloading, and no passive bridges) [12];
8. Cementation [13].

Based on the surface roughness, it has been proposed to categorize implants as (Albrektsson and Wennerberg 2004) [14]:

- smooth S_a < 0.5 µm;
- minimally rough S_a: 0.5–1.0 µm;
- moderately rough S_a: 1.1–2.0 µm;
- rough S_a > 2.0 µm.

S_a values have to be interpreted with care, because there are other parameters that characterize implant surfaces.

Literature reveals that rough surface implants (i.e., titanium plasma sprayed; TPS), once exposed to the oral cavity, have a higher tendency to develop peri-implantitis than minimally rough implants.

Moreover, some studies assess that rough implant surfaces are more susceptible to peri-implantitis establishment and progression [15].

However, several authors have indicated that frequent patients recalls are involved in peri-implantitis prevention, and a close recall scheme is essential [2,4–6].

Once peri-implantitis is established, laser-assisted treatment seems favored in literature [16]; various wavelengths are used: diodes (from 450 nm to 980 nm), Nd:Yag (1064 nm), Nd:Yap (1340 nm), and Er:Yag (2940 nm).

Lasers could be applied alone or in combination with a photosensitizer; photodynamic therapy (PDT) is the combination of light with a chromophore and oxygenated tissues. The goal of PDT is to deliver singlet oxygen to all tissues affected by pathogenic bacteria. If the wavelength used is absorbed in the chromophore used, laser ray cannot penetrate more in deeper, and if the power density is too low (usually PDT protocols use LLLT (low level laser therapy) energy, in order to avoid any thermal damage), the decontaminating effects are not enough. Recently has been proposed that a combination between high power and high frequency of diode laser 980 nm (using really high peak power combined with a low average power, in order to reduce thermal effects) and hydrogen peroxide 10 volume 3%, or modified hydrogen peroxide 10 volumes 3% [16–23]. This treatment modality was named "photodynamic therapy without dye" (OHLLT; oxygen high level laser therapy) in the protocol proposed by Gerard Rey in 1999 [24].

OHLLT technology is a therapy based on the combination of a penetrating laser with a modified and stabilized H_2O_2 10 vol. 3% solution. Several in vitro studies showed bactericidal activity of laser irradiation combined with hydrogen peroxide on numerous bacterial species. A comparative study on the effects of laser alone and combined with H_2O_2 10 vol. 3% showed these results [21–23]: (1) laser used alone produces poor results in the elimination of bacterial species involved in periodontal disease, (2) H_2O_2 used alone produces little effects in micro-organism elimination, and (3) laser combined with

hydrogen peroxide shows an antibacterial action that is much more effective on most of the microorganisms involved in periodontal disease. Laser energy activates the modified H_2O_2 solution, releasing free radicals and singlet oxygen that has antibacterial activity on Gram-positive and Gram-negative periodontal pathogens. The photochemical effect of this photodynamic therapy consists of activation of a photosensitizer (in this case hydrogen peroxide 10 vol. 3%), with a monochromatic beam—a laser beam characterized by a single wavelength (980 nm diode). The interaction between this photosensitizer and the laser produces photochemical reactions in which the energy acceptor is oxygen. The stabilized hydrogen peroxide contains oxygen, and its presence allows the reactions of photoactivation and production of singlet oxygen. The singlet oxygen is an oxygen-free radical that determines bacterial cells death (destruction of bacterial membrane, degradation of lysosomal membrane, alteration of mitochondrial function, and denaturation of DNA molecules) [17,19,21–23].

The aim of our study is to investigate, retrospectively, if a meticulous, laser-assisted, maintenance protocol could reduce the risk of peri-implantitis onset, including with the use of rough dental implant surfaces.

2. Materials and Methods

The present pilot study was approved by the Ethics Committee of the School of Medicine and Surgery at the Milano Bicocca University, (protocol n. 11/17), and derived from the approval of Italian National Institute of Health (ISS), protocol 30 July 2007-0040488, and it was conducted in accordance with the Declaration of Helsinki.

In this study we evaluated implants with rough surfaces, to assess their clinical performance over a period of years.

In particular, we considered two types of rough surface:

- Ti-Unite™ implant surface: This surface was introduced in 2000 (Nobel Biocare, Gothenburg, Sweden). It is characterized by a moderately rough thickened titanium oxide layer; the productive process leads to a duplex oxide structure through spark anodization in an electronic solution, that results in an outer barrier with numerous pores (depth between 4 and 10 microns) and an inner barrier layer without pores; moreover, it enhances osteoconduction, with faster anchorage to bone matrix, thanks to its high crystallinity and phosphorus content [25] [Figures 1 and 2].
- Ossean® implant surface: Ossean® is a moderately rough Ti–Al–V surface obtained through the resorbable blasted medium (RBM) process, followed by the incorporation of a low amount of CaP.

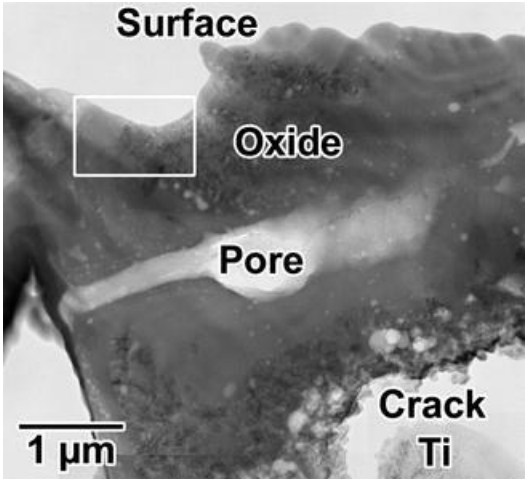

Figure 1. TiUnite™ implant surface: low-magnification transmission electron micrograph. Reproduced with permission from [26], Copyright Springer, 2015.

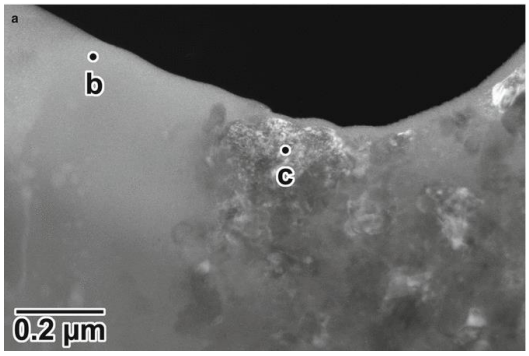

Figure 2. TiUnite™ implant: dark-field transmission electron micrograph of the depression on the surface. Reproduced with permission from [26], Copyright Springer, 2015.

The surface roughness was designed to obtain micro and nano irregularities, which should enhance implant biocompatibility compared to traditional surfaces, increasing the available contact surface and potentially improving the mineralizing cell attachment and expansion [27] [Figure 3].

The incorporation of a low amount of CaP may improve the surface biointeractivity during the initial osseointegration processes through the apatite nucleation on its surface without the abovementioned detrimental effects; however, no information on the apatite nucleation ability of the implant has been reported [28].

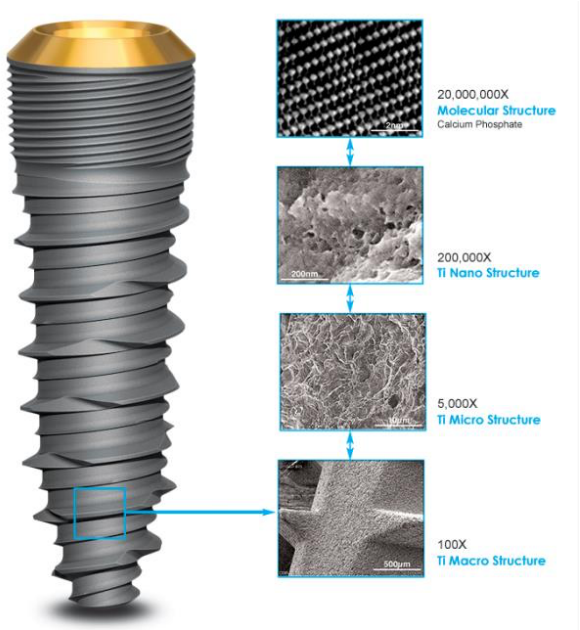

Figure 3. Ossean® implant surface [29].

For the selection of implants, we established the following inclusion criteria: both male and female, non-smoking history, absence of allergies, absence of uncontrolled systemic disease, absence of pregnancy or lactation, absence of abuse of alcohol or drugs, and acceptance of the maintenance protocol and laser-assisted treatments proposed.

Exclusion criteria were presence of allergies, presence of uncontrolled systemic disease, presence of pregnancy or lactation, abuse of alcohol or drugs, and non-acceptance of the maintenance protocol and laser-assisted treatments by signing an informed consensus.

Our sample consisted of 67% male patients and 33% female in the Ti-Unite group of implants; for the Ossean group of implants we selected 45% male patients and 55% female patients.

The average mean age of patients that received Ti-Unite implants was 61 years, while the average mean age for Ossean implants was 55 years.

In our study we analysed a total of 500 implants for the Ti-Unite surface, and 1000 implants for the Ossean surface.

Implants with the Ti-unite™ surface—273 in the upper arch and 227 in the mandible—had been inserted, for 65% of patients, in regenerated bone (PRF (platelet-rich fibrin), 45%; Bio-Oss®, 45%; and autogeneous bone, 10%); 179 in the upper arch with regenerated bone and 146 in the mandible with regenerated bone [Table 1]. All implants had a follow-up period of more than 10-years. (Rey 2018).

Table 1. Number of implants with Ti-Unite surface and Ossean surface, both in pristine and regenerated bone.

IMPLANTS	SUPERIOR	INFERIOR	TOTAL
Implants with Ti-Unite surface %superior/inferior	273 54.6%	227 45.4%	500
Implants with Ti-Unite surface in regenerated bone %superior/inferior	179 55.08%	145 44.92%	325
Implants with Ossean surface %superior/inferior	556 55.6%	444 44.4%	1000
Implants with Ossean surface in regenerated bone %superior/inferior	351 57.12%	288 42.88%	639

There were a total of 1000 implants with the Ossean® surface, 556 in the upper arch and 444 in the mandible. For 63.9% of patients, this type of implant had been inserted in regenerated bone; 351 in the upper arch and 288 in the mandible (Table 1). All implants had a follow-up of more than 10-years. (Caccianiga 2018).

All patients agreed with the following maintenance home hygiene protocol:

- Sonic brush with vertical movement (Broxo), interdental brushes, and oral irrigators (Broxo) at least two times every day Figures 4–6.
- Evaluation of subgingival plaque sample with analysis by contrast-phase microscopy every 4 months for all patients with implants, from the beginning until more than 10 years, in a close recall scheme (Figures 7 and 8).

Figure 4. Oral hygiene devices: seven devices for optimal domiciliary hygiene procedures. The most important are: 1, Sonic brush with vertical movement; manual toothbrush; 2. interdental brushes; and 3, oral irrigators.

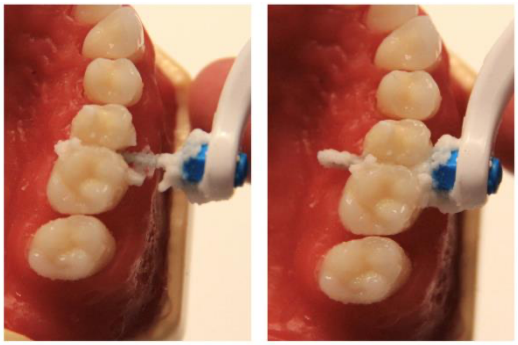

Figure 5. Interdental brushes in action.

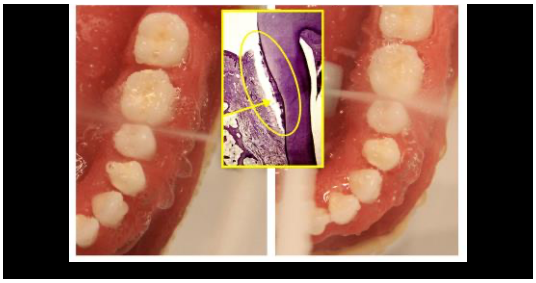

Figure 6. Oral irrigators in action, in order to remove sub-gingival biofilm.

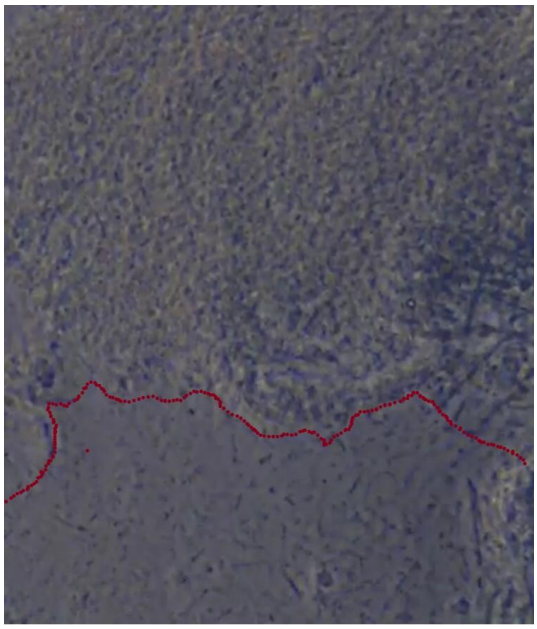

Figure 7. Contrast phase microscope with dividing line between compatible bacterial flora (above) and non-compatible bacterial flora (below).

Figure 8. Contrast-phase microscope in periodontal office.

In particular, due to the contrast phase microscopy results, we established the following protocol:

- ✓ In the presence of compatible bacterial flora (static flora, Gram-positive bacteria): usual periodontal supportive therapy with visits every 4 months;
- ✓ In the presence of non-compatible bacterial flora (spirochete, moving flora): immediate treatment with supra-gingival and sub-gingival ultrasonic instrumentation, air flow with bicarbonate powder, and a one-stage session of photodynamic therapy without dye (OHLLT) in the whole mouth and not only in peri-implant sites. This protocol has been applied even in the absence of signs of inflammation.

The one-stage session of photodynamic therapy without dye (OHLLT) consisted of:

1. Irrigation of periodontal and or peri-implant pockets with H_2O_2 10 vol. 3% or SIOXYL+ solution;

2. Aspiration of H_2O_2 10 vol. 3% or SIOXYL+ solution emerging from the gingival sulcus and leaving the remaining solution inside the pocket for 2 min;

3. Introduction of the HF Diode Laser 980 nm, Fiber 400 microns (Wiser Doctor Smile) within the pocket and reaching the bottom, radiation of subgingival tissues with a movement back and forth using the dedicated program, 60 s per side (2.5 W peak power, high frequency, 10 KHz, power average 0.5 W).

With regard to Sioxyl solution, a stabilized hydrogen peroxide 10 vol. 3%, Caccianiga et al. [18] showed that "the use of high-frequency lasers (LII) combined with hydrogen peroxide stabilized with glycerol phosphate complex (HP- GP), provides optimal results for a substantial decrease of the bacterial load combined with a maximal biostimulation induction of soft tissues and osteogenesis".

Later, patients were redirected to domiciliary protocol with sonic brush (Broxo), interdental brushes, and oral irrigator (Broxo) at least two times every day.

At 1 month follow-up: if in presence of compatible flora, patients returned to normal 4-month controls; if in presence of non-compatible flora, remotivation to domiciliar oral hygiene, and a one-stage session of laser-assisted PDT without dye.

This scheme was to be proposed unless refused by the patient. All patients agreed with this maintenance protocol.

Bone level measurements were recorded from implant neck to bone crest, both in the mesial and distal side of implants. For each implant under consideration the lowest measurement between mesial and distal aspect recorded. Measurements were made by the same operator.

The software used to record the measurements was Soredex Digora®.

These data were used in order to identify cases of peri-implantitis, establishing peri-implantitis diagnosis with MBL (marginal bone level) $\geq$ 3 mm, in accordance with Berghlund et al. [4].

Statistical analysis was conducted in order to compare bone loss in the upper arch or in the mandible due to peri-implantitis, for both Ti-Unite and Ossean surfaces.

Moreover, a second statistical analysis was carried out to compare bone loss due to peri-implantitis in regenerated bone and in pristine bone, for both Ti-Unite and Ossean surfaces.

According to the Kolmogorov–Smirnov test, overall data followed a normal distribution (value of the K-S test statistic = 0.0902; $p = 0.36804$).

Statistical analysis with Student's t-test (t-test for independent variables) was performed in order to investigate the above-mentioned topics, with a significance level of $p < 0.05$.

3. Results

Implants with Ti-Unite™ surface underwent peri-implantitis in eight implants (1.6% of the total amount of implants); other causes of implant loss were prosthetic problems (six implants, 1.2% of the total), fractures (four implants, 0.8% of the total), and lack of osteointegration (two implants, 0.2% of the total). Therefore, considering only implant loss, the majority was due to peri-implantitis (40%), followed by prosthetic problems (30%), fractures (20%), and lack of osteointegration (10%). The total number of implants lost was 20 (Table 2).

Table 2. Ti-Unite implants lost.

Implant Loss	Prosthetic Problems	Fractures	Lack of Osteointegration	Peri-Implantitis	Total
Total	6	4	2	8	20
% of total	1.2	0.8	0.4	1.6	4
% lost	30	20	20	40	

Implants with Ossean® surfaces underwent peri-implantitis in 15 implants (1.5% of the total amount of implants); other causes of implant loss were prosthetic problems (11 implants, 1.1% of the total), fractures (5 implants, 0.8% of the total), and lack of osteointegration (5 implants, 0.5% of the total). Considering only implant loss, the majority was due to peri-implantitis (40%), followed by prosthetic problems (30%), fractures (15%), and lack of osteointegration (15%). The total amount of implants lost was 36 (Table 3).

Table 3. Ossean® implants lost.

Implant Lost	Prosthetic Problems	Fractures	Lack of Osteointegration	Peri-Implantitis	Total
Total	11	5	5	15	36
% of total	1.1	0.5	0.5	1.5	3.6
% lost	30	15	15	40	

In the Ti-Unite group of implants, two implants underwent peri-implantitis at the fourth year of follow-up, three in the seventh year, and three in the ninth year of follow-up.

In the Ossean group of implants, three implants underwent peri-implantitis in the fifth year of follow-up, six in the seventh year, four in the eighth year, and two in the tenth year of follow-up.

Regarding peri-implantitis that occurred in Ti-Unite implants, there was no statistical difference between the mean MBL in the upper arch (3.88 mm) and the mean MBL in the mandible (4 mm) ($p = 0.7795$) or between the mean MBL in regenerated bone respect (3.92 mm) and the MBL in pristine bone (3.9 mm) ($p = 0.9574$).

For peri-implantitis in Ossean implants, the statistical analysis conducted between mean MBL in the upper arch (3.63 mm) and mean MBL in the mandible (3.55 mm) showed no statistical differences ($p = 0.6973$). The MBL in regenerated bone (3.73 mm) and MBL in pristine bone (3.4 mm) also showed absence of statistical differences ($p = 0.1020$).

4. Discussion

In this study we analyzed the presence of implant failures, and in particular the number of implants lost due to peri-implantitis, in two different types of implant surfaces, Ti-Unite™ surface and Ossean®. Both surfaces present a common characteristic that is roughness of its surface.

A review by Wennerberg et al. (2018), analyzing a total of over 17,000 implants with at least 10 years of follow-up, showed a high survival rate of implants with Ti-Unite™ surfaces; in fact, this anodised surface showed the lowest probability of failure compared with other different surface modifications such as plasma-sprayed titanium [30].

A meta-analysis by Karl and Albrektsson (2017), based on 106 publications, reported a 10-year survival rate of Ti-Unite implants of 95.14%. Of these studies, 18% (19 studies) investigated the prevalence of peri-implantitis in Ti-Unite™ surface: in a total of 1229 patients, 64 underwent peri-implantitis, with a prevalence of 5.2% and a mean follow-up of 47.89 months [31].

In contrast, Simion et al., in 2017, reported a considerably higher percentage of peri-implantitis with anodized implant surface (28.3% of the implants evaluated) [29]; the same author, instead, reported a considerably lower percentage of peri-implantitis with machined implant surfaces (1.8% of all the implants examined) [32].

Our findings are in contrast with the results obtained by Simion et al. regarding rough surfaces [33]; in fact, we observed 1.6% of peri-implantitis with Ti-Unite™ surface and 1.5% of peri-implantitis with Ossean® surface.

This conflict could be explained by differences in classifications of peri-implantitis: only a few authors pay specific attention to the role of bacteria's role in peri-implantitis etiology; Renvert et al. suggested a classification including the research of specific bacteria such as *Porphyromonas gengivalis, Prevotella intermedia, Fusobacterium nucleatum, Tannerella forsythia, Aggregatibacter actinomycetencomitans*, and *Treponema denticola* [34–36].

Moreover, Esposito et al. stated that there is no evidence of a major risk of peri-implantitis due to bacterial contamination related to rough surfaces, without differences in the prevalence of peri-implantitis in 5-year and 10-year follow-up data between smooth and rough surfaces [37,38].

In the non-surgical therapy of periodontal diseases the persistence of specific subgingival species including *A. actinomycetemcomitans, P. gingivalis,* and *T. denticola* (their ability to invade the subjacent periodontal tissues) has been associated with poor response to treatment by scaling and root planing [39]. Also peri-implantitis have been associated with specific micriobiota that make it difficult to remove with non-surgical therapy alone [40].

Swider et al. [15] in a recent meta-analysis evaluated 49 articles but only 7 met the strict inclusion criteria regarding quality assessment. The studies included those by Birang et al. [41], Caccianiga et al. [16], Persson et al. [42], Arisan et al. [43], Yoshino et al. [44], Bassetti et al. [45], and Dörtbudak et al. [46] who concluded that "a high-power diode laser may have some effect on peri-implant pathogens causing peri-implantitis, whereas Er:YAG laser application shows no significant effect on oral bacteria in the long term. aPDT (antimicrobial photodynamic therapy) has the ability to reduce the total count of the different bacterial strains associated with peri-implantitis, e.g., *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. denticola*, *T. forsythia*, *F. nucleatum*, and *C. rectus*".

In addition, outcomes of surgical/regenerative treatment are strictly associated with the presence of high bacterial load and specific pathogen complexes in deep periodontal pockets (also associated with intra-bony defects) [47]. In peri-implantitis, instead, surgical

therapies aim at improving implant surface cleanability and modifying bone and soft tissue morphology to reach a possibility of new osseointegration [40].

Our clinical approach was inspired by these assumptions, leading to a systematic approach with a strict follow-up of patients, in order to intercept early signs of mucositis and/or peri-implantitis and in order to anticipate the onset of laborious clinical conditions.

Moreover, we established that patients with implants must be strictly followed up with a plaque sample every 4 months, in order to assess quantity and quality of bacteria, individualizing the treatment for the specific patient, keeping under control potential clinical problems.

Statistical analysis conducted reveals that there were no statistical differences between mean MBL in the upper arch or the mandible or between pristine bone or regenerated bone, for both kinds of implant surfaces; these results highlight the importance and influence of our protocol (laser-assisted treatment and modified maintenance protocol) because we can notice a very likely absence of influence of implant positioning (upper or lower arch) or bone quality (pristine or regenerated bone) in peri-implantitis onset.

As a result, we can state that our good results in regard to the low percentages of peri-implantitis that occurred in our findings derive from an optimal management protocol of patients consisting of three main characteristics:

- continuous microbiological control with scanning electron microscope;
- constant domiciliary oral hygiene remotivation with adequate instruments for subgingival plaque control;
- use of photodynamic therapy without dye (OHLLT), as proposed by G. Rey, in order to obtain an efficacy toward red and orange complexes of Socransky, even before the insurgence of clear signs of peri-implant diseases; this protocol of early and preventive treatment, with OHLLT, has proved to be efficient in decreasing most of the periodontal and peri-implant pathogens (a property that is not evident in Erbium laser because of its less deep tissue activity) [16–23].

Further consideration could be given to studying the importance of prevention, microbiological control with plaque samples, appropriate home oral hygiene protocols, and efficacy of PDT without dye (OHLLT) in anticipating potential periodontal and peri-implant problems.

5. Conclusions

Implants with rough surfaces have proved to be effective in implant-prosthetic rehabilitations with more than 10 years of follow-up; in fact, considering our results, these kinds of surface, with a close scheme of maintenance protocol, both at home and in the periodontal office, showed a very low percentage of peri-implantitis. The use of phase-contrast microscopy could aid the periodontist in early detection of microbiological pathologic conditions with an increase of Gram-negative micro-organisms in order to prevent mucositis and peri-implantitis with a laser-assisted approach.

Future randomized and controlled studies are needed to confirm the results of the present retrospective analysis.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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