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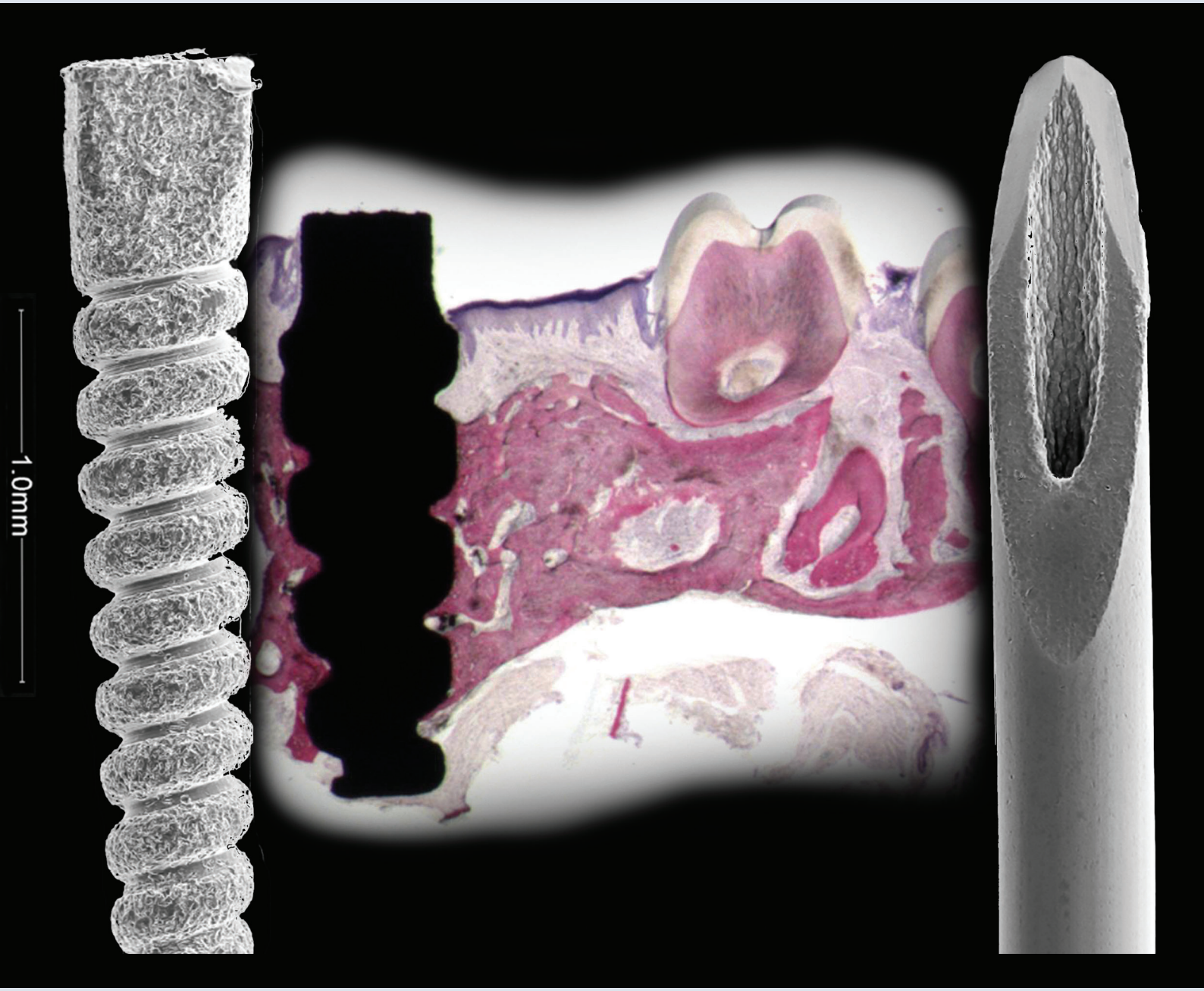

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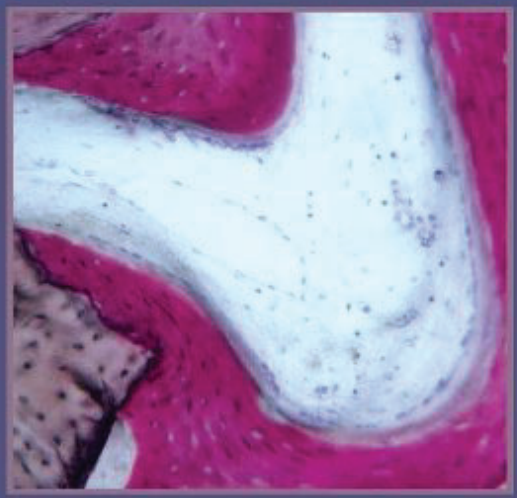

Bone Response to Dental Implant Materials

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ORIGINAL ARTICLE

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Peri-implant alveolar bone resorption in an innovative peri-implantitis murine model: Effect of implant surface and onset of infection

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Abstract

Purpose: To compare the difference in alveolar bone resorption around implants after immediate placement in a bacterial induced experimental periimplantitis murine model. The various conditions that were examined were: Effect of implant surface characteristics and the onset of the induced infection.

Materials and Methods: Screw-shaped titanium implants, smooth-surface or sand-blasted large-grit acid-etched (SLA) coated, were inserted immediately after extraction of the first upper left molar, in 90 5-6-week-old BALB/c mice. The mice were infected with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* 21 (early infection) or 42 days (delayed infection) after implantation. Six weeks post infection, bone volume around inserted implants was measured using micro-CT, and was compared to alveolar bone level around teeth. Histological analysis was also performed.

Results: The level of bone loss was significantly higher around the implants compared to the teeth, for smooth surface implants the bone loss was higher than of the SLA surface in both control and infected groups with no statistical significance. The survival rate of the implants in immediate infection was 75% compared of the 100% survival of the delayed infection and control mice. There is no significant difference between the early and the delayed infection in alveolar bone loss level around the implants.

Conclusions: This model can assist in studying the differences in alveolar bone resorption in different implants and their effect on the development of the disease.

KEYWORDS
compact fan-beam-type computerized tomography, delayed infection experimental peri-implantitis, dental titanium implant, murine model, oral mixed infection in mice, SLA coated, smooth surface

1 | INTRODUCTION

Dental implants have revolutionized dentistry. Implant-supported prostheses have become the first treatment of choice for their wide variety of treatment options. When oral implants are placed and restored according to accepted protocols, the implant success rate can reach to 95% for over 10 years.[1] Since 1983, when Branemark released his extensive research findings, the number of dental implants placed is increasing constantly. Today, under care and according to indications, insertion of dental implant seems to represent a "safe" treatment

Einat Varon-Shahar and Ariel Shusterman contributed equally to this work.

option,[2] and although no reliable documentation exists, it has been estimated that ~12 million osseointegrated oral implants are placed annually in the world[3] according to the American academy of implant dentistry (AAID), each year the number will grow in 500 000 in the US alone.

Although in many cases dental implants have been reported to achieve long-term success, they are not resistant to infection and subsequent complications. Dental implants and adjacent tissue are susceptible to inflammatory diseases such as peri-implant mucositis and peri-implantitis. Peri-implantitis, an emerging disease characterized by an inflammatory process around implants, includes both soft tissue inflammation and progressive loss of supporting bone beyond biological bone remodeling.[4] The bone loss around implants varies from 11 to 47% in subjects, depending on the threshold used.[5] Today, there is no effective treatment protocol for peri-implantitis, primarily because of incomplete knowledge of the pathogenic mechanisms of this disease.

Biopsies from human peri-implantitis and periodontitis lesions show that the two diseases have many features in common.[6,7] Peri-implant diseases have been associated with Gram-negative anaerobic bacteria, similar to those found around natural teeth in patients with severe chronic periodontitis.[8-11] A number of risk factors have been identified that may lead to the establishment and progression of peri-implantitis.[12] These include poor plaque control, residual cement, smoking, and diabetes, along with previous periodontal disease. The history of peri-implantitis has been associated with a higher prevalence of peridontitis. Peri-implantitis was detected more than twice as frequently in periodontally-compromised than in periodontally-healthy subjects. It has also been reported that the maintenance of periodontal health, rather than a previous history of periodontitis, is the critical determinant of an increased risk of peri-implantitis.[13,14] Systematic reviews[15-17] have indicated that although the implant survival rate may not be affected by the periodontal history, peri-implantitis was more frequently found in patients with a history of periodontitis.

Although sharing similarities with periodontitis in the bacterial initiators and the key immune components, the rate of disease progression, the severity of inflammatory signs and the histopathology of peri-implantitis may be different.[18] The micro-biome studies also showed microbiologically distinct ecosystems in the different pathologies.[19] Recently, it was also suggested that the marginal bone loss around implants is the result of a provoked foreign body reaction.[3]

Interestingly, patients who already had one removed implant following periimplantitis and failure of the implant are 1.3 times more likely to have a second implant removed, indicating that failure is dependent on systemic and/or genetic factors,[20] and that implant complications tend to be clustered in a subset of individuals rather than being randomly distributed among the population.[20-27]

It is not clear whether the host's genetic susceptibility determines the susceptibility to biological complications of the dental implant, even though it has been suggested as one of the potential risk indicators.[28] The association between IL-1 gene polymorphism and peri-implantitis remains to be determined, as there are conflicting findings.[29] A systematic review[30] analyzing 27 relevant articles found no consensus among the studies. Another systematic review,[31] questioning the association between genetic predisposition and dental implant biological complications, methodological and study design issues, restricted the possibility to draw robust conclusions.

Recent study[32] tried to find the molecular differences between peri-implantitis and periodontitis at the transcriptome level, comparing the gene expression of affected tissues from both phenotypes. Interestingly, the results presented in this study indicate only a few similarities between peri-implantitis and periodontitis, indicating that peri-implantitis and periodontitis are different disease entities with shared as well as distinct features.[32]

Animal models are an important tool to demonstrate periodontal disease. In the recent years a murine model was published demonstrating ligature induced peri-implantitis.[33] This model successfully investigates the oral pathogens, which cause the disease, and managed to imitate the natural occurrence of peri-implanttis onset and progression in human. The implant model as was published by Pirih[34] included an extraction of the first upper molar tooth in mice, followed by an insertion of a titanium implant after a healing period of 8 weeks. The implant was smooth surface, 1 mm length, and 0.5 mm wide, and was inserted to the alveolar bone by using a full thickness flap technique.

The induction of the disease can also be demonstrated in several techniques. Baker[35] and Polak[36] used the gavage technique to challenge the mice by *periodontal* bacteria to induce periodontitis, while Pirih induced infection by injection of LPS that were produced from *P gingivalis* to the mucosa around the implant.[34] The use of a silk ligature around the implant as a platform for accumulation of oral pathogens was also published as a successful method to induce peri-implantitis.[33,37]

Here, we propose a murine model that has been developed in our laboratory based on the implant insertion by Mouraret[38] and Baker's alveolar bone loss model.[35,36] This is an innovative model demonstrating for the first time mixed infection induced alveolar bone loss around an immediate implantation after extraction in a murine model. To initiate peri-implantitis, we used mixed infection with the two anaerobic bacteria *P gingivalis* and *F nucleatum*.[36] The phenotype was measured as the residual alveolar bone volume, using microCT scans, which were shown to provide an accurate quantitative measurement of bone loss around murine teeth.[39-41] This murine model will provide a tool to compare the pathogenic process of peri-implantitis and periodontitis comparing the bone loss around teeth and implant in the same mice with no environmental or genetic differences as contributing cofounders. In addition, the effect of implant surface treatment and the infection timing will be evaluated in this model.

2 | MATERIALS & METHODS

2.1 | Mouse populations

All experimental mice and protocols were approved by the Institutional Animal Care and Use Committee of HUJHH (approval number: MD-14029-4), which adhere to the Israeli guidelines which follows the NIH/USA animal care and use protocols.

Ninety mice were divided to four different groups: The early infection group included 58 mice and the delayed infection group included 20 mice. Those groups were subdivided to control vs infectious group. The group that tested the differences in alveolar bone loss between sand-blasted large-grit acid-etched (SLA) surface coated implants and smooth surface implants contained 12 mice (six mice received SLA coated implants and six received smooth implants). All the 12 mice received early infection.

2.2 | Bacterial cultivation

P gingivalis strain ATCC 33277 and *F nucleatum* strain PK 1594 were grown in peptone yeast extract containing hemin and vitamin K (Wilkins Chalgren broth, Oxoid Ltd, UK), in an anaerobic chamber with 85% N_2, 5% H_2 and 10% CO_2, followed by three washes in phosphate-buffered-saline (PBS). The bacterial concentration was spectrophotometrically standardized to OD_{650}nm = 0.1 for *P gingivalis*, corresponding to 10^{10} bacteria/ml[2], and OD_{660}nm = 0.26 for *F nucleatum*, corresponding to 10^9 bacteria/ml.[3] This step was necessary to count the amount of bacteria, which were grown. Before the infection, the two bacteria were mixed together. The ratio was 1:1 (*Pg*: *Fn*), and 10^9 CFU were used then.

2.3 | The implant

Implants used in this study are titanium threaded having a diameter of 0.5 mm and a length of 1.5 mm. These mini-implants were made by Ditron Dental and have undergone the following multistage surface treatment process—Al_2O_3 surface blasting, double acid etching, high purity cleaning procedures as used for commercial implants for human. The surface treatment process was validated based on scanning electron microscope, Energy-dispersive X-ray spectroscopy, and X-ray photoelectron spectroscopy testing (Figure 1). The smooth surface implants used in this study were fabricated in the same conditions with no additional surface treatments.

2.4 | Implant placement

The mice were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (16 mg/kg). After rinsing the animals' mouths with a povidone-iodine solution for 1 minute, the first molar tooth was extracted and the self-tapped implant was placed manually in the socket of the root (immediate implantation), as described in detail by Mouraret.[38] The implant was inserted by the use of a special conductor until stability of the implant in the socket was achieved. Following surgery, clinical examinations was performed and the mice received subcutaneous injections of buprenorphine (0.05-0.1 mg/kg) for pain relief once daily for 3 days (Figure 2).

2.5 | The oral infection model

All mice were infected according to the mixed infection model described by Polka,[35,36,42] The infected mice were used for two different infected groups: early infection—21 days following the implantation and delayed infection—42 days following the implantation. The mice were given sulfamethoxazole (Resprim) 10 mL/ per 500 mL in water ad libitum for 10 days followed by a 3-day washout (antibiotic-free period). The mice were then superinfected with a 400 μL mixture of *P gingivalis* (10^9/ml) and *F nucleatum* (10^9/ml) in PBS with 2% carboxymethycellulose by gavaging three times at 2-day intervals. The mice were examined daily and body weight was tested once a week. The experiment was terminated 42 days after the last gavage. Maxillae was harvested and fixed in 4% paraformaldehyde for histology, or used for microCT analyses (See Figure 3 for schematic model of the experiment).

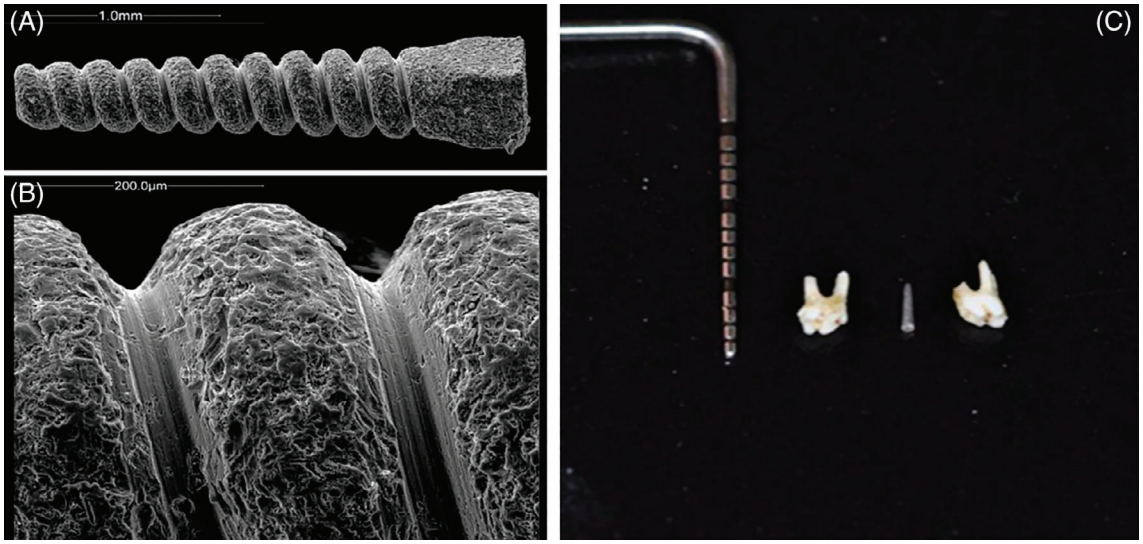

FIGURE 1 The mini-implant that was specifically designed for the study. The implant was specifically designed for our research. The same surface treatment and sterilization pathways were done (Al2O3 surface Blasting, Double Acid Etching and High purity cleaning procedures). Implant images A (magnification × 79) and B (magnification × 1400) were captured with SEM. C, A comparison between the mouse teeth and the implant, scale according to dental probe

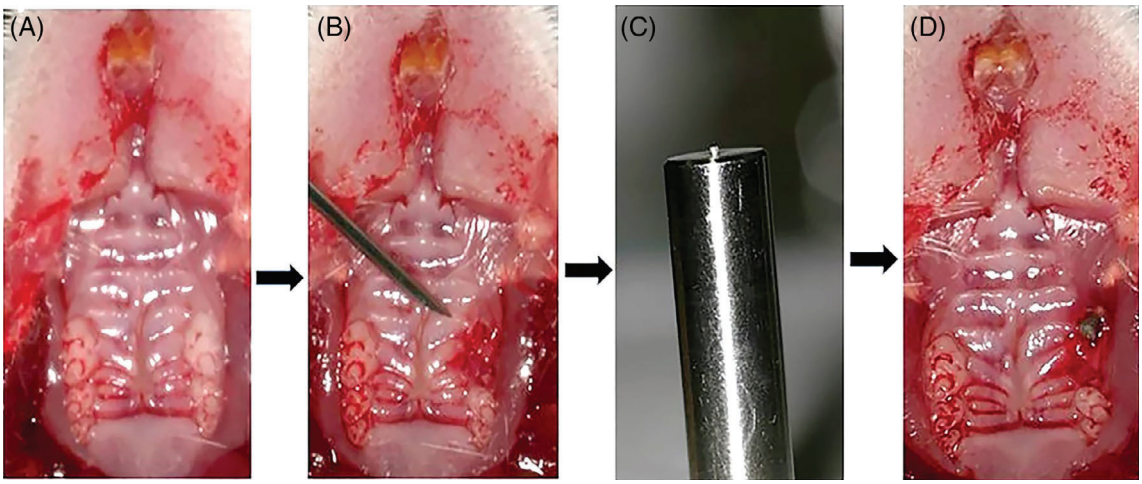

FIGURE 2 Extraction site and implantation. A, Mouse upper jaw showing 3 M on each side. B, Left side of upper jaw showing extraction site of first molar. C, Implant on special conductor. D, Implant with primary stability in socket of extracted tooth

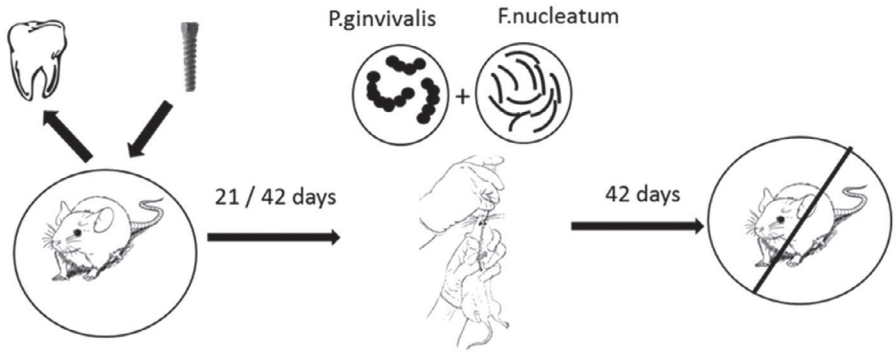

FIGURE 3 A schematic model of the experiment

2.6 | Micro-CT analysis

Maxillary hemi-jaws were analyzed by compact fan-beam-type tomography (μCT 40, Scanco Medical, Bassersdorf, Switzerland) for quantitative three-dimensional (3D) analysis of the alveolar bone. Samples were placed in a cylindrical sample holder, the sagittal plane of the specimen was set parallel to the x-ray beam axis, and about 300 microtomographic slices at increments of 12 μm were acquired covering the entire bucco-palatal width of each hemi-jaw. Image segmentation of bone, dentin, enamel, and pulp were obtained by applying a manually selected threshold for all the specimens (Figure 4). A reference line was set throughout the microtomographic slices at a set distance from the coronal part of the implant and the residual alveolar bone adjacent to the implant, thus measuring the vertical alveolar bone loss around the implant. The defect in the bone was measured at 40 sites around the implant −20 sites for the distal part of the implant and 20 sites for the mesial part. The results will be presented as the residual bone in mm, as a linear measurement.[39]

The alveolar bone loss around teeth was measured as bone volume loss around the second upper molar. After determining the reference line, the volume of the alveolar bone was measured at a distance of 260 μm below the CEJ of the tooth. The amount of alveolar bone volume was measured at 10 sites around the mesio-buccal root and at 15 sites for the disto-buccal root. The results will be presented as the residual bone in mm^3.

2.7 | Histology

The specimens were washed in saline solution and immediately fixed in 10% buffered formalin, and processed for histology. The specimens were processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned, along its longitudinal axis, with a high-precision diamond disk at about 150 μm and ground down to about 30 μm with a specially designed grinding machine. The slides were stained with acid fuchsin and toluidine blue. The slides were observed in normal transmitted light under a Leitz Laborlux microscope (Leitz, Wetzlar, Germany).

2.8 | Data analysis

Was performed using a statistical software package (SigmaStat, Jandel Scientific, San Rafael, California). One-way analysis of variance (ANOVA)

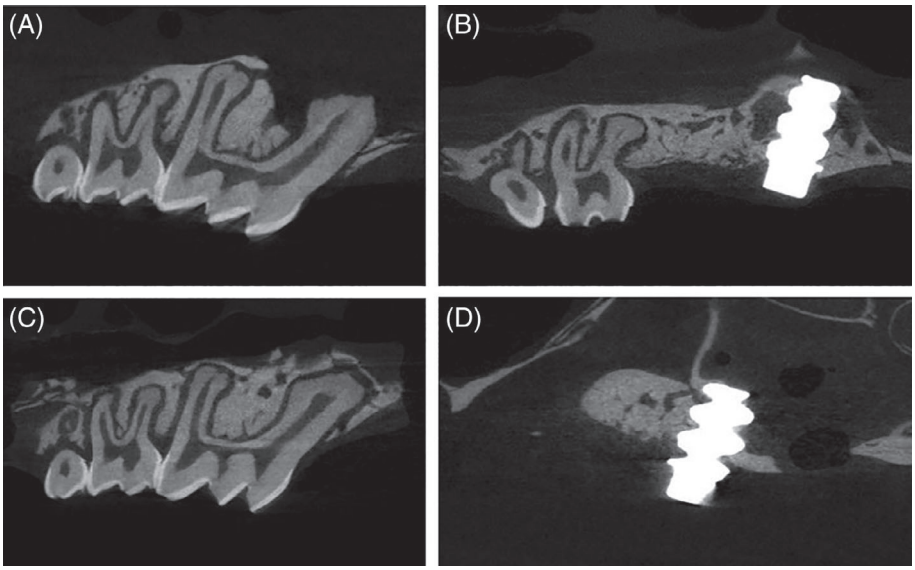

FIGURE 4 MicroCT image of Right hemi-maxilla reconstructed by micro-CT. Figures show a buccal view of mouse hemi-maxilla, reconstructed by microCT. Only hard tissue (calcified tissues or metal) can be seen after reconstruction by the microCT. (A + B) A control mouse; the titanium implant (white) is completely surrounded by alveolar bone (gray). The alveolar bone level is adjacent to the CEJ of the third molar tooth. (C + D), An infected mouse; vertical bone resorption (probably due to bacterial infection and inflammatory bone resorption) can be seen from the occlusal platform of the implant apically compared to the control. The infection is also shown in the first molar tooth

was used for testing the significance of the difference between the treated groups. If significance was established, the inter-group differences were tested for significance according to the *t*-test with the Student-Newman-Keuls correction for multiple testing. The level of significance was determined at $P < .05$. All the results are presented as mean values ± SE of the mean.

3 | RESULTS

3.1 | Histological evaluation

The socket healing was observed at all sites and osseointegration could be seen at all mice. Alongside the whole implant, there was compact bone.

In the slide, representing the control group (Figure 5)—The maximum height of the crest was observed close to the first thread of the implant. Bone tissue was present around the implant starting almost from the top of the implant. On one side of the implant, the newly-formed bone was in close and tight contact with the implant surface. This bone was compact, lamellar with few marrow spaces. No gaps were present at the interface. No epithelial downgrowth, connective tissue, or foreign body reaction cells were present. No inflammatory cell infiltrate was present. The top of the implant was covered by a keratinized mucosa with an underlying fibrous, connective tissue with no signs of inflammation.

Test group (early infection) (Figure 6)—Newly-formed bone is found at the level of the second thread. This bone is in close contact with the implant surface all around the implant perimeter. Few small marrow spaces were present in the peri-implant bone. The apical portion of the implant was covered by connective tissue, with no signs of inflammation. In the coronal portion, at the interface with the first thread, it was possible to observe connective tissue covered by a keratinized mucosa. No epithelial downgrowth was present.

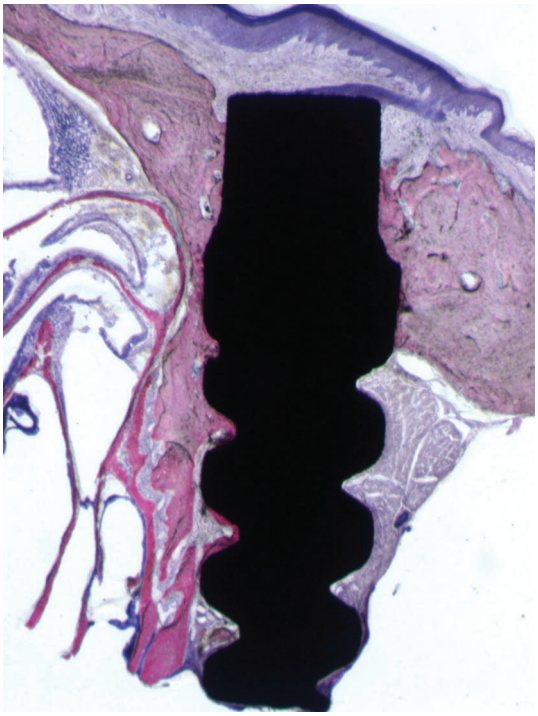

FIGURE 5 Histological specimen of an implant from the control group. The bone is found almost to the top of the implant. Acid fuchsin-toluidine blue × 25

In the slide, representing the delayed infected group (Figure 7)—The maximum height of the crest was observed close to the second-third thread of the implant. Bone was present around the implant starting from the third thread toward the apex. This bone was in close contact with the titanium surface, with no gaps at the interface. No epithelial downgrowth was present. A few small marrow spaces were present in a peri-implant location. The coronal portion of the implant was lined by a connective tissue, covered by a keratinized epithelium.

3.2 | Experimental peri-implantitis

3.2.1 | Implant survival rate: Early vs delayed infection

The survival rate of the implants was calculated as the relative survival percentage in each experiment compared to the survival rate of the implants in the control group. Twenty-two of 28 implants from the control group survived which comprise the key to comparison as 100%. Eighteen implants of 30 in the early infection group survived (75%), and 20/20 implants survived in the delayed infection group (Figure 8).

3.2.2 | Alveolar bone loss evaluation in early and delayed infection

The level of alveolar bone loss around the infected teeth was 16.9 mm^3 compared to 7.85 mm^3 in the control group (Figure 9A).

The mean alveolar bone loss around the infected implants in early infection was 31.39 mm^3 compared to 11.5 mm^3 in the control group. This difference is statistically significant (Figure 9B).

The mean alveolar bone loss around the infected implants in the delayed infection was 37.8 mm^3 compared to 8.86 mm^3 in the control group. This difference is statistically significant too (Figure 9C).

3.2.3 | Alveolar bone loss evaluation: Early compared to delayed infection

Evaluating the effect of the infection timing on the severity of bone loss around the implants, we found that the alveolar bone loss following early and delayed infections is similar with no effect of the infection timing. The level of bone loss was not statistically significant between the two infected groups. The existence of the infection caused bone loss around the implants regardless the level of the osseointegration of the implants (Figure 10).

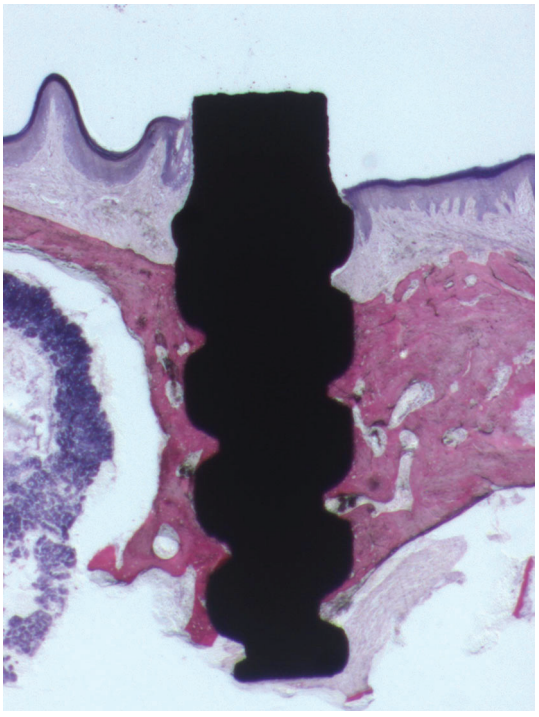

FIGURE 6 Histological specimen of an implant from the infected group (Early infection). Newly-formed bone is found at the level of the second thread. Acid fuchsin-toluidine blue × 25

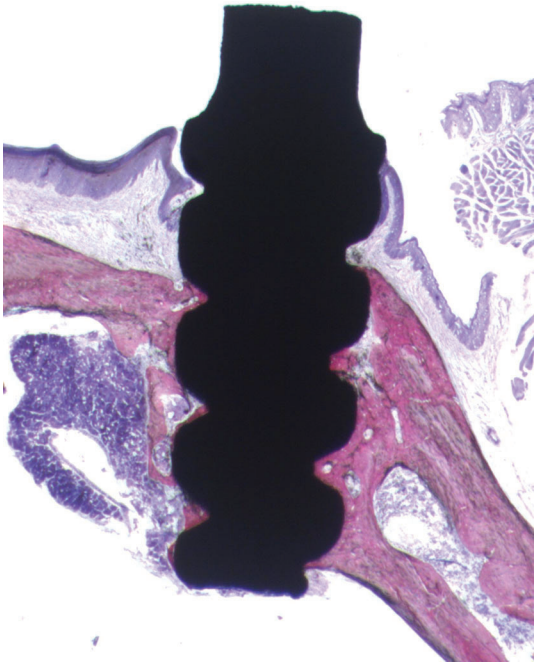

FIGURE 7 Histological specimen of an implant from the infected group (Delayed infection). Bone is found at the level of the second-third thread. Acid fuchsin-toluidine blue × 25

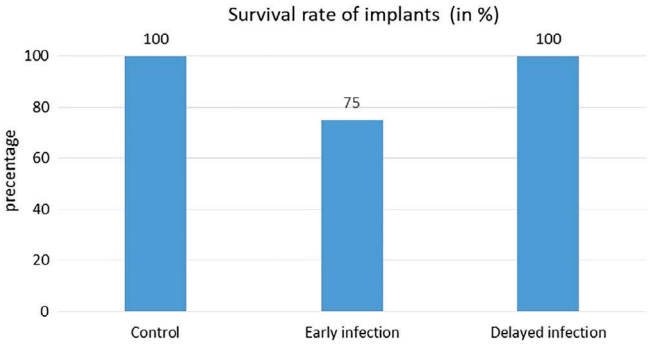

FIGURE 8 Survival rate of implants in the early and delayed infection vs control. One hundred percentage of all the implants in the control and delayed infection group survived, 75% of all implants in the early infection group survived

3.2.4 | Alveolar bone loss evaluation: SLA treated surface implants compared to smooth surface implants

All the smooth surface implants in the control group survived the early infection (6/6) while in the infected group four of six implants survived (66%).

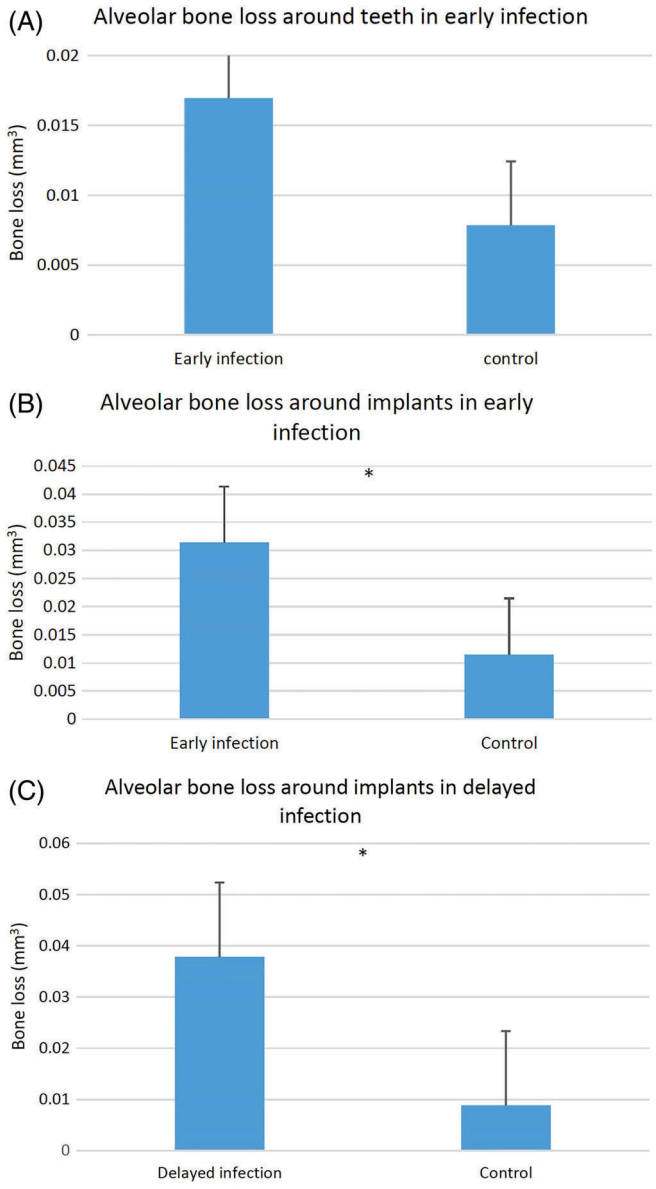

FIGURE 9 Alveolar bone loss around implants and teeth in early infection and around implants in delayed infection. A, In the early infection group, the alveolar bone loss around teeth was 16.9 mm^3 compare to 7.85 mm^3 in the control group. B, In the early infection group, the alveolar bone loss around implants was 31.39 mm^3 compare to 11.5 mm^3 in the control group. C, In the delayed infection group, the alveolar bone loss around implants was 37.8 mm^3 compare to 18.86 mm^3 in the control group

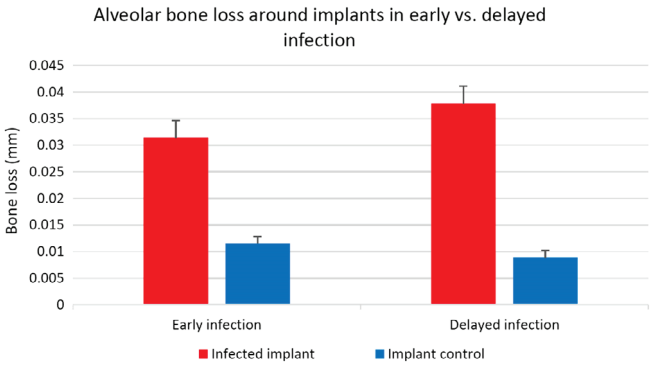

FIGURE 10 Alveolar bone around implants in early vs delayed. Infection in the Delayed infection group, the level of alveolar bone loss around the infected implants was 37.8 mm^3 compare to 29 mm^3 in the immediate infection group

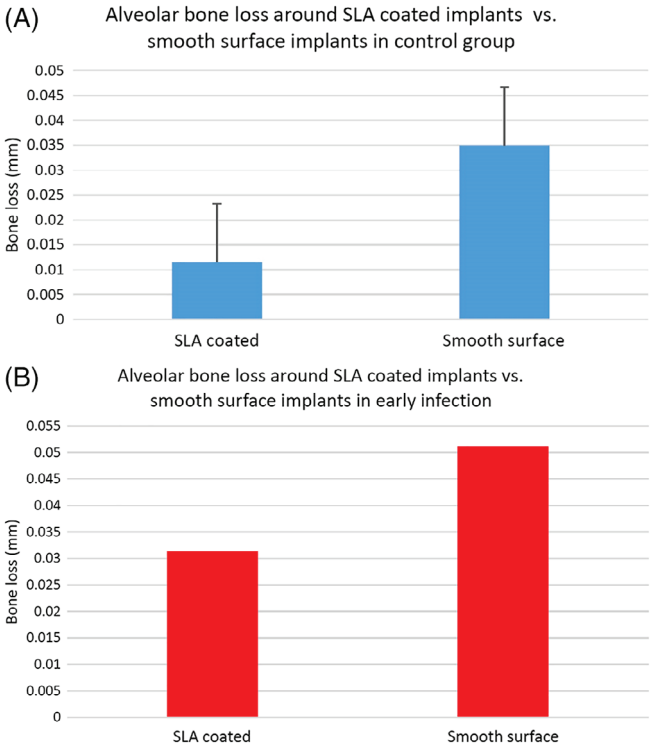

FIGURE 11 Alveolar bone loss around SLA coated implants vs smooth surface implants. A, In the control group, the alveolar bone loss around SLA coated implants was 11.5 mm^3 compare to 34.9 mm^3 around the smooth surface implants. B, In the early infection group, the alveolar bone loss around SLA coated implants was 31.39 mm^3 compare to 51.2 mm^3 around the smooth surface implants

The level of alveolar bone loss around the smooth surface implants in the control group was higher, 34.9 mm^3 compared to 11.5 mm^3 in the SLA coated implants (Figure 11A).

The level of alveolar bone loss around the infected smooth surface implants was 51.2 mm^3 compared to 31.39 mm^3 in the SLA coated implants (Figure 11B). The results show a higher loss of alveolar bone compare to the SLA coated implants.

4 | DISCUSSION

The human studies of prei-implantitis have their limitations. Histological examinations and genetic investigations are limited, as well as the background of individual's systemic condition that is hard to asses. Animal's studies must be combined to solve the pathogenesis of the disease or even suggesting treatment protocols.

Several recent studies suggest different murine experimental peri-implantitis models. Some of them suggest delayed implantation by waiting to tissue healing following extraction as a prior condition to the implantation, and others suggest implantation in the palate. Trang & Nguyen[33] demonstrated a successful model of inducing peri-implantitis in a murine model by the use of silk ligature. In his research, the implant was inserted 8 weeks after extraction of the first upper molar and peri-implantitis was induced by application of a silk ligature around the implants necks 4 weeks after implantation. In this model, the ligature increased plaque retention and consequently induced inflammation. Takamori[43] demonstrated an onset of peri-implantitis on an immediate implanted implant in rats by the use of intraperitoneal lipopolysaccharide (LPS) from *Escherichia coli* (*E.coli*) and a topical application of LPS in the palatal sulcus of the implant. Pirih[34] also published a successful model of inducing peri-implantitis in mice, by the use of injection of *P gingivalis* LPS to the peri-implant soft tissues for 6 weeks. These both studies used LPS, which is well-known irritant of the immune response as an inducer of inflammation, but is different from the natural process of periimplantitis. Tzach-Nahman,[44] published an immediate implantation of an implant in the palatal socket of an extracted molar, the infection was induced by gavage of *P gingivalis* with a vehicle of CMC.

Our experimental peri-implantitis is unique in several aspects: first—the implant is inserted in its proposed placed at the socket place of the natural tooth in the maxillary alveolar ridge, and not at the center of the palate as was described by earlier publications[32] The palatal area is composed of cortical bone while the alveolar ridge is a trabecular bone. This bone quality difference is important for model characterization and for clinical relevance.[33]

Second, the immediate insertion of the implant prevents the need for a second surgical procedure which may lead to an increase in mortality rates. Third, the inflammation was induced by a mixed infection of two gram negative bacteria (*P gingivalis* and *F nucleatum*) by gavage. This is a well-established model of oral infection by our group[39,40] and very easy to manipulate compared to ligature induced or intragingival injections as suggested in other models. In our model we used a mixed infection which was shown previously[40] as mimicking the situation in periodontal infections. The disadvantage of the ligature technique for experimental peri-implantitis is that it is not specific and add a mechanical parameter that is not existing in natural periimplantitis in human.[45] Another technique that is mentioned to induce peri-implantitis is the use of LPS injections. This method can bypass the bacterial colonization process, allowing focusing on the inflammatory components of the disease, and avoiding variations that are inherently associated with bacterial colonization disease models.[46] Yet the main disadvantage in this technique is that it requires another manipulation in the oral cavity, and is given twice a week for 6 weeks.[34] The gavage technique is an easy way for oral challenge, given only three times for a week period and does not require any manipulation on the implantation site. The results from our study showed a significant effect of the inflammation induced by gavage technique in the form of alveolar bone loss around the teeth and implants (Figure 9).

Our new model inducing minimal trauma during the tooth extraction and implant insertion, and the immediate implantation mimics the original use of implants and reduces the time of the study (from 24 to 12 weeks 5, 6). The implant design and configuration is similar to that used for human by the same manufacture and same process. The same surface treatment and sterilization pathways were done and the study design supports the clinician's workflow.

This design enables us to avoid determining the end-point for fixture insertion. The bone resorption is measured related to the adjacent tooth compared to the baseline level of the crestal bone. This is a fixed point giving us a secure point for measurement and comparison between the implants and thus providing a more reliable data for comparison and analyzing.

Implant survival rate—Our results showed a remarkable success in the survival of the implants in the delayed infection, none of the implants failed, similar to the survival rate of the control group. These survival rates were high even without the acceptable waiting period of 8 weeks after the tooth extraction and before the implantation, as is accepted in the literature. In our histological findings and in the measurements of alveolar bone levels we proved that the implants were well integrated in the bone of the tooth extraction site (Figures 4–7). As a model, we found it easier to place the implants at the socket, even for young students without the experience of implant insertion procedures; no flap designs or drilling is needed, and no additional tools are required. This simplification of the model reduced implant contamination by bacteria and increased the successful rate of implants.

The relatively low survival rate in the early infection implant group might be because of an insufficient time for the implant to complete the osseointegration process in the bone in an infected environment. While in the delayed infection, the bone had enough time to complete the osseointegration process and even yet, in the presence of a severe infection the implants were still stable despite the signs of alveolar bone loss that were evident in the tissue surrounding them.

Alveolar bone loss—The amount of alveolar bone loss was well demonstrated while comparing the control teeth and implants to the infected teeth and implants, respectively. As expected, the infected teeth showed a higher alveolar bone loss due to the exposure to the periodontitis induced bacteria. The loss of bone levels in the infected implants may indicate a similar reaction of inflammation around the implant, and validates this model as a model to induce peri-implantitis disease in the presence of specific bacteria.

The amount of alveolar bone loss around the infected implants was significant in the early as well as in the delayed infection group. These findings establish the further experiments, which compared the implant reaction to the infection in these two different exposure times.

4.1 | Early vs delayed infection

Our hypothesis was that inducing the disease after a longer period, 42 days since the implantation, would prove that the successful osseointegration process had happens, and that would be

demonstrated in less alveolar bone loss around implants. Yet we discovered that the contrary had happened, although the results were not statistically significant, the alveolar bone loss was higher in the delayed infection implants instead in those with the early infection (Figure 10). A possible explanation for that is that there are other factors, which can affect the clinical signs of peri-implantitis and the progress of the disease around the implants. Now that the model was proven to be efficient another consideration regarding the genetic influence must be taken into consideration when decoding this disease. And this is a subject for further investigation.

4.2 | Smooth vs SLA coated implants

Contrary to the early implants which were generally smooth with minimal surface irregularities, most of the current implants surfaces are rough and have irregularities. The surface roughness is intended to enhance cellular activity during early healing and increase the bone-implant contact, thus to deliver better osseointegration.[47] Summarizing the results of several studies showed sufficient evidence that titanium implants with a micro-rough surfaces achieve a faster bone integration, a higher percentage of Bone Implant Contact and a higher resistance to shear documented with higher removal torque values when compared with titanium implants with a polished or machined surface.[48,49]

However, a debate is still present regarding the difference in their survival rate. In a retrospective study that compared the survival rate of over 2000 implants (rough vs smooth) there was no significant difference in the survival rate between the two groups.[47] The same conclusion was presented in Esposito study[50] while the only two implants that failed in this study were machined surface. In our study, the survival rate of rough surface implants was higher (75%) in compare to that of smooth surface (66%). When comparing the alveolar bone loss in the control group of smooth vs SLA coated implants, the results are even more explicit and a higher amount of bone loss was evident in the smooth surface implants, probably due to poor osseointegration from the beginning.

In the presence of infection, the surface type of the implant has a different effect on the interaction of bone to implant. The adhesion of osteoblasts is enhanced on rough surfaces, and they are being more active in the remodeling of a new bone in the implantation site, thus may cause a higher absorption of bone around the implant.[51] In the SLA implants in our study, the osseointegration process was relatively successful, which lead to a lower rate of alveolar bone loss in the control group, yet after the infection, the total ratio of bone loss was twice as high in compare to the control SLA group. On the other hand, the total ratio of bone loss in the smooth surface implants was only 1.5 higher in the infection compare to the control group. Despite the successful rate of osseointegration in the SLA implants compared to the smooth implant, the alveolar bone loss ratio was higher in the presence of infection in the SLA implants and not in the smooth surface as should have been expected, due to poor initial osseointegration. The reason to this difference should be further examined as it may be an indicator to the presence and influence of factors from the immune response of the body to the presence of foreign body that encouraged the resorption. The bone to implant contact cannot be addressed as the sole factor in long term survival, Instead implant survival is likely to be related to a multitude of factors that effects the performance of the implant.[47] The discovery of further mechanism which influences the alveolar bone resorption must be more investigated to further discover the inflammation as a part of peri-implantitis.

By the use of this model, we can study the effect of implant materials, design, and topography, we can study the pathogenesis of the disease from both bacterial and host response, as well as genetic predisposition to periimplantitis using multiple genetic induced inbred mice. This model could also be used to study new approaches for preventive and therapeutic protocols to achieve prevention and treatment of the disease. Further research is still required in this field. This research reviled the necessity of following the progression of the disease in different time intervals. One possible way of doing so is by the use of the imaging technique on live mice and not only at the end of the disease. Thus, we will be able to closely monitor the deteriorating of the disease and maybe try to intervene before the disease is far advanced until the failure of the implant.

Our results support the protocol of tooth extraction, immediate implantation, and bacterial challenge for inducing experimental peri-implantitis in mice. This minimal invasive and easy to handle protocol resemble the same sequence of events in humans and could contribute to the disease understanding.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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