Comparison of Surface Treatments of Endosteal Implants in Ovariectomized Rabbits

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Purpose: The aim of this work was to evaluate osseointegration of endosteal implants with two different surface treatments at early stages (~3 weeks) in the tibia of healthy and ovariectomized rabbits. Materials and Methods: The study comprised 10 adult New Zealand female rabbits (Oryctolagus cuniculus; 6 months and 3.0 ± 0.5 kg). Five animals were subjected to bilateral ovariectomy to mimic osteoporotic-like conditions, and the remaining rabbits (n = 5) served as the healthy control group. After 3 months, specimens from the ovariectomized and control groups were subject to implant placement in both tibiae, using two different types of surface treatment. A total of 36 implants were placed, n = 18 acid-etched and n = 18 anodized. After 3 weeks, euthanasia of the animals was performed, and samples were obtained for processing. Bone-to-implant contact and bone area fraction occupancy were quantified to evaluate the osseointegration parameters around the implant surface and within the thread area, respectively, and nanoindentation tests were performed to determine elastic modulus and hardness of the new bone. Both analyses were performed on the entire implant (total), as well as individually within the cortical and bone marrow cavity area. Results: All animals were evaluated with no signs of infection or postoperative complications. The total bone-to-implant contact and bone area fraction occupancy results, independent of surface treatment, yielded significant differences between the ovariectomized and control groups (P = .002 and P < .001, respectively). In the narrow cavity, analyzing the surface treatments independently as a function of bone condition, the only differences detected were in the anodized treatment (P = .04). Regarding the elastic modulus, differences were detected only with the anodized implants between the ovariectomized and control groups (P = .015). Conclusion: At 3 weeks after implant placement, there were better osseointegration values of the implants in the healthy control group compared with the ovariectomized group independent of surface treatment. Also, specifically in the medullary region of the rabbit tibia, the acid-etched implants had more uniform osseointegration values in conditions of low-quality bone in comparison to the anodized implants, histomorphometrically and biomechanically. Int J Oral Maxillofac Implants 2021;36:38–46. doi: 10.11607/jomi.8459

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Titanium endosteal implants are the most common, reliable, predictable, and versatile device employed for the rehabilitation of edentulous patients, yielding favorable survival rates.1/2 Currently, a wide variety of dental implants, each having respective and different characteristics in terms of geometry (ie, shape, length, diameter, thread design, eg, distance between threads, among others) and surface finishing. The macrogeometry is the primary factor affecting primary stability, initial mechanical interlocking upon engagement with bone (osteotomy wall) at the time of placement. Once the osteotomy is prepared and the implants are inserted, a series of biologic steps ensues, ultimately achieving osseointegration, defined by Brånemark as the “direct structural and functional connection between the living bone and surface of a load-bearing implant.”3 The series of events commences with a large number of cellular elements coming into direct contact with the implant at the interface, triggering the process. The mechanisms are influenced by the implant surface: chemical composition and/or microgeometry roughness, as the cells exhibit different behavior when
comparing smooth and rough surfaces. A large quantity of literature and research has been dedicated to the study of the effect of surface treatments; whether chemical or physical, surface features can be tailored/tuned, such as machined or treated (ie, sandblasting, acid etching, anodizing, plasma spray, laser treatment, and deposition of nanoparticles).

The literature specifies that implants with rougher surfaces (ie, acid etched) have better osseointegration and survival rates than machined implants without surface treatment. This increased osseointegration observed with acid-etched implants is attributed to surface morphology and surface energy, which positively influences the reaction between the interface of the endosteal implant surface and bone during the regeneration/remodeling process. One of the most common acid etching treatments is subjecting the implant to a solution of HCl + H2SO4, HF + HNO3, and HNO3. The acid etching process controls the size and porous distribution on micrometer and nanometer scales, modifying parameters such as etching protocol, concentration, and temperature, which produces an attractive surface for cellular elements (ie, osteoblasts). As demonstrated by Choi et al, there is an increase in cell attachment with an increased surface roughness. While acid etching is commonly employed, other alternatives to modify surfaces are available, such as anodizing, an electrochemical process where the implant is immersed in an electrolyte solution with an actively applied current, resulting in a porous surface structure. Anodized surfaces have been demonstrated to be superior in comparison to unaltered, machined implants. One of the most notable differences observed was contact angle and wettability. Higher contact angles, yielding increased wettability, and osteogenic cell interaction with the implant surface have been well documented.

While the literature indicates that chemical and physical alterations/modifications to the implant surface yield increased levels of osseointegration and survival rates, the majority of research studies have focused on specimens or patients presenting with "healthy bone." However, taking into consideration the aging world population compounded with the increasing elderly population having access to implant-assisted rehabilitation, it is of significance to understand the behavior and potential impact of different surface treatments on the remodeling process, ie, secondary stability, specifically in low-quality bone, commonly found in patients diagnosed with osteoporosis. Osteoporosis, a generalized disease of the skeletal system, characterized by bone loss and deterioration of the microarchitecture of bone tissue, compromises bone resistance and results in lower mechanical strength, greater bone fragility, and susceptibility to brittle fractures. Additionally, these patients presenting with excessive bone resorption by osteoclasts coupled with an inadequate response by osteogenic cells to increased resorption during bone remodeling is common.

Osteoporosis may also affect the mandible, primarily by decreasing the height of the alveolar ridge, density of trabecular or cancellous bone, and mandibular cortical width, which renders it a low-quality substrate for endosteal implant placement. Due to the unfavorable consequences, different strategies, such as different surgical techniques, implant designs, and surface treatments, have been developed and/or explored to achieve more predictable degrees of osseointegration in osteoporotic conditions. However, thus far, there has been no consensus in the literature with respect to the survival rate of endosteal implants with different surface treatments in patients diagnosed with osteoporosis, as indicated by some systematic reviews.

Some of the most recent meta-analyses indicate that there are no differences after 1 year of implant placement regarding survival rates, but the studies do highlight the low quantity and high risk of bias of the available articles. It should also be noted that fewer studies can be found specifically evaluating protocols involving immediate or early loading of implants, which makes it important to further develop strategies or evaluation of different implant surfaces that potentially may behave better in early stages of the osseointegration process, which could allow immediate or early loading of these endosteal implants. Therefore, the aim of this work was to evaluate osseointegration of endosteal implants with two different surface treatments, at early stages (~3 weeks) in the tibia of healthy and ovariectomized rabbits.

**MATERIALS AND METHODS**

**Implants**

The study utilized 36 internal hexagon connection implants (Conexao, Centro Industrial Arujá) with dimensions of 4.0 mm in length by 2.0 mm in diameter. The implants comprised two different surface treatments, anodized (n = 18) and acid-etched (n = 18), previously characterized by Elias et al (Table 1).

**In Vivo Model**

The study utilized the medial region of the rabbit tibia model due to its low-density characteristics because of the presence of an extended medullary cavity as well as its length, allowing maintenance of an appropriate distance between implants during the placement, minimizing the risk of bone fracture, while minimizing the number of animals. The study was conducted according to the Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines.
Surgical Procedures
The study comprised 10 adult New Zealand female rabbits (*Oryctolagus cuniculus*; ~6 months and 3.0 ± 0.5 kg), which were acquired from and allowed to acclimate at the Center of Excellence in Morphological and Surgical Studies (Faculty of Medicine, Universidad de La Frontera, Temuco, Chile). Prior to any surgical intervention, the animals were randomized by individual cages by a veterinarian who did not participate in the study. Five of the 10 rabbits were subjected to bilateral ovarioctomy to mimic an osteoporotic-like state induced by estrogen deficiency, and the remaining rabbits (n = 5) served as the healthy control group.

Ovariectomy and Implant Placement
All surgeries were performed under a sterile field and general anesthesia with ketamine (40 mg/kg) and xylazine (5 mg/kg) intramuscularly with continuous monitoring of the vital functions. After the induction of anesthesia, the skin area was shaved and disinfected with povidone iodine, with an incision made to access the abdominal cavity, where both ovaries were ligated and isolated with resorbable 4.0 suture to prevent bleeding and subsequently resected. The site was closed with layer-by-layer suturing with absorbable suture (polyglycolic acid) 4.0 in the deepest areas and in the muscle, while skin closure was completed with 3.0 silk. After the procedure, the ovariectomized subjects were allowed to heal, and the osteoporotic-like state was allowed to progress for 3 months following the bilateral ovariectomy.

After the 3-month gestation period, the ovariectomized and control rabbits were subject to the subsequent surgical protocol for implant placement, performed by another single surgeon (M.P.). After induction of general anesthesia, the medial regions of both tibiae (left and right) were shaved, skin was disinfected with povidone iodine, an incision was made with a no. 15 scalpel blade, and a mucoperiosteal flap was lifted with the help of a molt periosteal elevator, to expose the bone. Subsequently, with a 1.5-mm-diameter carbide conical surgical bur, the osteotomy was performed at 1,200 rpm with abundant and continuous irrigation of saline solution. Once the osteotomy was completed, the implants were inserted, with an insertion torque between 30 and 35 Ncm, followed by the placement of the corresponding healing cap. Lastly, the site was closed in a layer-by-layer fashion, with a resorbable suture (polyglycolic acid) 4.0 for the muscle tissue and 3.0 silk for the skin.

After surgery, the animals were placed in individual cages with ad libitum access to water and food and 12-hour light/dark cycles. The postoperative care included the administration of enrofloxacin (5 mg/kg) every 24 hours for 3 days, along with diclofenac sodium (80 mg/kg) intramuscularly once a day for 3 days.

After 3 weeks (21 days) of follow-up, euthanasia of the animals was performed according to the approved protocol (overdose of sodium thiopental), and implant/bone samples were obtained by blunt dissection, immersed, and stored in 70% ethanol.

Histologic Processing
Specimens of implants with surrounding tissues were subjected to nondecalcified histologic processing. The bone-implant blocks were gradually dehydrated in a series (70% to 100%) of ethanol solutions and subsequently embedded in a methyl methacrylate-based resin. Embedded blocks were then cut into sections using a low-speed diamond saw (IsoMet 2000, Buehler). The sections were glued to slides and ground on a grinding machine (MetaServ 3000, Buehler) under water irrigation with a series of SiC abrasive paper (Buehler) until they were ~100 μm thick. The samples were then stained in Stevenel blue and Van Gieson to differentiate the hard and soft tissues.

Histomorphometric Analysis
The histologic slides were qualitatively and quantitatively analyzed using histology micrographs and image analysis software (ImageJ, National Institutes of Health). Bone-to-implant contact and bone area fraction occupancy were quantified to evaluate the osseointegration parameters around the implant’s surface and within the thread area, respectively. Bone-to-implant contact quantifies the degree of osseointegration by tabulating the percentage of bone contact over the entire relevant implant surface perimeter, while bone area fraction occupancy quantifies bone growth within the implant threads as a percentage. Both analyses were calculated with the help of the software Leica Application Suite (Leica Microsystems). Both analyses were performed by a single, blinded user (L.W.). Both analyses were performed of the entire

<table>
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<th>Ra</th>
<th>Rz</th>
<th>Rq</th>
<th>Rt</th>
<th>Rv</th>
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Table 1 Mean Values of Roughness Parameters for Both Surface Treatments
implant, as well as individually within the cortical and bone marrow cavity area (Fig 1).

Nanoindentation
Slides for nanoindentation were prepared with a similar methodology as previously described (histomorphometry section), with an additional step of subjecting slides to 1200 polishing paper, as well as a series of monocristalline diamond suspension solutions (Buehler), from 9 µm to 0.1 µm, in an effort to eliminate any residual scratches.

The imaging and indentation processes were performed, at room temperature, using a nanoindenter (950 Ti; Hysitron) equipped with a Berkovich diamond three-sided pyramid probe. A load profile previously described by Witek et al²⁴ was utilized, consisting of a maximum load of 300 μN applied at a rate of 60 μN/s, followed by a dwell time of 10 seconds and an unloading time of 2 seconds, generating a load-displacement curve.

The nanoindentation testing was performed at two sites: (1) cortical bone and (2) marrow cavity bone, with each region of interest, cortical and marrow cavity, respectively, receiving nine nanoindentations, with a separation of 5 µm in the x- and y-axis. The generated load-displacement curve ultimately yielded elastic modulus and hardness of the tested bone.

Statistical Analysis
Sample size was determined a priori using power analysis based on preliminary experiments. The analysis revealed that n = 10 rabbits (5/group) was sufficient to achieve power > 80% to detect any potential differences in bone-to-implant contact and bone area fraction occupancy (α was set to .05).

The histomorphometric and biomechanical result data are presented as mean values with their corresponding 95% confidence interval (mean ± 95% CI). Prior to any statistical analysis, the data were submitted to the Shapiro-Wilk test for data set normality, followed by statistical analysis using a general mixed model analysis (IBM SPSS version 23 software, IBM). The histomorphometry results (bone-to-implant contact and bone area fraction occupancy) comparisons were evaluated as a function of animal health: healthy vs ovariectomized. The nanoindentation results, similar to the histomorphometric results, were made between the values of hardness and elastic modulus, as a function of group and type of implant.

RESULTS
At the end of the in vivo experimentation, all animals in the study were evaluated with no indications of any signs of infection or postoperative complications.

Qualitative Histology
The histologic micrographs, in the specified region of interest of cortical bone, depicted greater amounts of lamellar bone in contact with the peri-implant surface compared with the marrow cavity independent of surface treatment (anodized and acid-etched) both in the control group (Figs 2a and 2b) and in the ovariectomized group (Figs 2c and 2d). When evaluating the marrow cavity in terms of the newly regenerated bone, a disorganized and immature pattern was observed for both groups, with greater amounts of new bone being observed in the acid-etched surface treatment group in comparison to the anodized treated implant.

Histomorphometry Results
The total bone-to-implant contact results, independent of implant surface treatment, yielded significant differences between the ovariectomized and control groups (P = .002), with significantly lower mean values observed in the ovariectomized group (42.04% ± 9.86% and 29.73% ± 11.98%, for the control and ovariectomized groups, respectively; Fig 3a). Comparing implant surfaces (anodized vs acid-etched) as a function of animal health (healthy and ovariectomized), no statistical differences were detected (Figs 4b and 4c). Analyzing implant surfaces independently as a function of bone condition (ovariectomized vs healthy), there were significant differences in both types of implants, anodized (P = .01) and acid-etched (P = .04; Fig 4a). Further evaluation in terms of the region of interest, bone-to-implant contact at cortical bone, did not yield statistical differences (Figs 3b and 4b), whereas in the marrow region, bone-to-implant contact yielded significant differences, but only between animal health, for ovariectomized vs control groups independent of surface treatment (P = .04; Fig 3c).

Evaluation of total bone area fraction occupancy followed a similar trend to total bone-to-implant contact, where statistical differences were detected between
ovariectomized and control groups (P < .001; Fig 5a) with no differences between implants as a function of bone condition. Analyzing implant surfaces independently as a function of bone condition, a statistical difference was detected for both surface treatments (Fig 4d; anodized, P = .001; and acid-etched, P = .03). Further evaluation in terms of the region of interest, bone area fraction occupancy results in the cortical bone region, did not yield any significant differences (Figs 4e and 5b), whereas the marrow region of interest, bone area fraction occupancy, did show significant differences between ovariectomized and control (P = .04; Fig 5c). When analyzing the implant surface treatments independently as a function of bone condition, the only differences detected were in the anodized treatment (P = .04), while the acid-etched yielded statistically homogenous results (Fig 4f).

**Nanoindentation**

Analysis of the elastic modulus yielded significant differences in the cortical bone for the anodized treated implant when evaluated as a function of health conditions (P = .038; Fig 6a), while the acid-etched treated implant yielded no statistical differences. For the results of elastic modulus in the marrow cavity, when making a comparison between both implant surfaces, significant differences were observed between them in the control group (P = .039; Fig 6b). The mean values of the anodized surface treatment were higher in comparison to the acid-etched surface treatment, while in the ovariectomized group, the values were higher in the acid-etched group, albeit with no statistical differences. However, analyzing the surface treatments independently as a function of bone condition, differences were detected with the implants subjected to
the anodized surface treatment \((P = .015)\), while the acid-etched surface treatment did not yield significant differences (Fig 6b). Regarding hardness, there were no significant differences in the cortical or marrow cavity region (Figs 6c and 6d).

**DISCUSSION**

The osseointegration of implantable devices (ie, titanium dental implants) in the host tissue occurs through a series of events, mediated by various factors. Micro- and nanoscale dental implant surface treatments are one of the parameters that have the greatest influence on the success rate of the treatment.\(^{11}\) The implants utilized in this study were acid-etched and anodized; these surface treatments were performed according to what was reported by Elias et al.\(^4\) According to their scanning electron microscopy results, regarding surface morphology in the case of acid etching, they were able to report a homogenous surface with uniformly distributed microcavities with defined borders, and when observed with greater magnification inside these microcavities, they could report roughness on a nanometric scale, while in the anodized implant, they reported that the surface presented volcano-shaped saliences. On the other hand, with respect to roughness values, there were no significant differences between both surface treatments.

During the bone remodeling process, specifically in a healthy patient, there must be a balance between...
bone resorption by osteoclasts and bone formation by osteoblasts, a vital process for the osseointegration of dental implants.\textsuperscript{25} This balance is disrupted in patients with osteoporosis, where one of the primary causes is linked to estrogen deficiency, resulting in increased bone resorption by osteoclasts and a poor response of bone formation by osteoblasts.\textsuperscript{26} The literature is not well-defined as to whether all endosteal dental implants are associated with decreased degrees of osseointegration in a bone with osteoporosis compared with healthy bone. In a systematic review conducted by Dereka et al.,\textsuperscript{27} where different animal models of osteoporosis were analyzed with the main analysis being the percentage of bone-to-implant contact, they indicated a decrease in osseointegration levels under conditions similar to those of osteoporosis; still, they also indicated that solid conclusions cannot be reached due to the high heterogeneity and the low overall quality of the available studies.

The results of the present study yielded significantly lower values of total bone-to-implant contact and bone area fraction occupancy associated with the ovariectomized group compared with the control, which yielded a higher primary stability due to the intimate contact with the surrounding bone, thus resulting in these elevated and uniform values at the time point of 3 weeks, which does not necessarily imply that it is a result of bone growth as shown previously by Lahens et al.\textsuperscript{28}

The results in the medullary region of interest of the tibia indicated that neither implant outperformed the other in the ovariectomized group. However, the acid-etched implant displayed a more uniform behavior compared with the anodized implant. The literature is not clear regarding the superiority of one surface treatment over another in healthy subjects, but there is consensus that the osteogenic quality of the implants with some type of surface treatment is superior compared with machined implants.\textsuperscript{6} Nonetheless, current evidence regarding the behavior of different surface treatments in osteoporotic tissues is still limited.

From a biomechanical perspective, bone is a difficult tissue to analyze due to its viscoelastic characteristics and high heterogeneity.\textsuperscript{29} The results of the present study yielded discernable differences in the hardness and elastic modulus values between the cortical and marrow cavity region bone in relation to dental implants of both groups. This is not surprising considering that in the cortical region, due to the type of osteotomy, the greatest amount of bone present in the first 3 weeks was preexisting mature bone. At the marrow cavity, practically all of the existing bone was woven bone, and therefore disorganized and with lower mechanical properties compared with cortical bone.
In the evaluation of the elastic modulus, higher mean values indicate that the tissue had the capacity to withstand higher loads.\textsuperscript{30} The results of the present study showed that in the marrow cavity, when analyzing how both surface treatments studied behaved independently as a function to bone condition, they followed the same trend as in the histomorphometry, with significant differences reported with the anodized surface, while the acid-etched implant presented statistically homogenous values between groups. In other words, the newly formed bone in relation to the acid-etched implant would have the ability to withstand higher loads compared with that formed in relation to the anodized implant.

Recent studies have shown the development of different strategies in order to enhance osseointegration in low-quality bone conditions, eg, ultraviolet (UV) photofunctionalization of titanium,\textsuperscript{31} deposition of parathyroid hormone-related protein on the surface of titanium,\textsuperscript{32} and the evaluation of different surface treatments presenting promising results, such as the studies of Lotz et al and Momesso et al,\textsuperscript{33,34} showing higher degrees of osseointegration associated with rougher and hydrophilic surfaces in ovariectomized rats. Nonetheless, it is not easy to compare their results with those reported in the present study because of different animal models, and this work also analyzed characteristics of elastic modulus and hardness of the newly formed bone.

The present study has its own limitations, particularly the analysis with only one time point and a reduced sample size. Taking into consideration the addressed limitations, the results obtained indicate that in the early healing, the first 3 weeks after placement of the endosteal implants, as expected, there were better osseointegration values of the implants in the healthy control group compared with the ovariectomized group independent of surface treatment when analyzing the cortical and medullary region together. However, when analyzing the medullary region independently, the acid-etched implants behaved more uniformly in conditions of low-quality bone in comparison to the anodized implants, histomorphometrically and biomechanically, making the acid-etched implant a promising tool in low-quality bone conditions. However, further follow-up studies are recommended, particularly using a larger more translational model (ie, ovine) in an effort to better correlate/model a clinically equivalent environment (with respect to bone biology and biomechanics), as well as adding additional time points and comparing more surface treatments. Limitations aside, the findings presented provide a basis for the development of a potential future randomized controlled clinical trial, with the intention of improving the available evidence regarding immediate and early loading of endosteal implants in patients diagnosed with osteoporosis. A controlled clinical trial will be able to yield further valuable, clinically derived data, which will assist in decision-making regarding the surface treatment that provides the most predictable results in low-quality bone conditions.

CONCLUSIONS

At 3 weeks after implant placement, higher degrees of osseointegration were observed for implants in the healthy (control) group in comparison to the ovariectomized group, independent of surface treatment. Taking into consideration the conditions of low-quality and low-density bone, such as in the medullary region, the acid-etched implants presented a more uniform osseointegration in comparison to the anodized implants, which was confirmed histomorphometrically and biomechanically.

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