

Article

Effect of Osteoporosis on Well-Integrated Bone Implants

Amani M. Basudan ¹, Marwa Y. Shaheen ¹, Abdurahman A. Niazy ², Jeroen J.J.P. van den Beucken ³, John A. Jansen ³ and Hamdan S. Alghamdi ^{1,*}

¹ Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; abasudan@KSU.EDU.SA (A.M.B.); mashaheen@KSU.EDU.SA (M.Y.S.)

² Department of Oral Medicine and Diagnostic Sciences, College of Dentistry, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; aaniazy@ksu.edu.sa

³ Department of Dentistry-Biomaterials, Radboudumc, P.O. Box 9101, 6500HB Nijmegen, The Netherlands; Jeroen.vandenBeucken@radboudumc.nl (J.J.v.d.B.); John.Jansen@radboudumc.nl (J.A.J.)

* Correspondence: dalghamdi@ksu.edu.sa

Abstract: The installation of dental implants has become a common treatment for edentulous patients. However, concern exists about the influence of osteoporosis on the final implant success. This study evaluated whether an ovariectomy (OVX)-induced osteoporotic condition, induced eight weeks postimplantation in a rat femoral condyle, influences the bone response to already-integrated implants. The implants were inserted in the femoral condyle of 16 female Wistar rats. Eight weeks postimplantation, rats were randomly ovariectomized (OVX) or sham-operated (SHAM). Fourteen weeks later, animals were sacrificed, and implants were used for histological and histomorphometric analyses. A significant reduction in the quantity and quality of trabecular bone around dental implants existed in OVX rats in comparison to the SHAM group. For histomorphometric analysis, the bone area (BA%) showed a significant difference between OVX (34.2 ± 4.3) and SHAM (52.6 ± 12.7) groups ($p < 0.05$). Bone-implant contact (BIC%) revealed significantly lower values for all implants in OVX (42.5 ± 20.4) versus SHAM (59.0 ± 19.0) rats. Therefore, induction of an osteoporotic condition eight weeks postimplantation in a rat model negatively affects the amount of bone present in close vicinity to bone implants.

Keywords: dental implants; osseointegration; osteoporosis; ovariectomy; rat femoral condyle; animal model



Citation: Basudan, A.M.; Shaheen, M.Y.; Niazy, A.A.; van den Beucken, J.J.; Jansen, J.A.; Alghamdi, H.S. Effect of Osteoporosis on Well-Integrated Bone Implants. *Appl. Sci.* **2021**, *11*, 723. <https://doi.org/10.3390/app11020723>

Received: 23 December 2020

Accepted: 12 January 2021

Published: 13 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Dental implants are a successful treatment approach for edentulous patients [1,2]. The long-term success of dental implants is directly related to their ability for early osseointegration [3]. Osseointegration is a coordinated dynamic process involving cellular and molecular events that comprise a cascade of cellular and extracellular signaling pathways in a chronological sequence of different phases of bone healing, including inflammation, angiogenesis, osteogenesis and remodeling [4,5]. Implant osseointegration is strongly influenced by local and systemic factors, which are related either to the implant itself or to the patients' systemic status. Examples of these factors include bone quality and quantity, implant design, surface characteristics and the medical condition of the patient. For example, altered bone metabolism can negatively affect dental implant survival and function [6–8]. Indeed, the role of normal bone metabolism has been considered essential to maintaining a healthy bone structure and function around implants. The homeostasis of bone metabolism can be disrupted by an imbalance between bone resorption and formation due to bone diseases such as osteoporosis.

Osteoporosis is a systemic skeletal disease characterized by an imbalance between bone formation and bone breakdown, resulting in a dramatic change in the bone structure [9]. The associated clinical adverse effects are mainly reported to occur in the long

bones, vertebrae and the jaw bones [10]. Osteoporosis is highly prevalent among post-menopausal women as a result of estrogen deficiency [11]. Additionally, osteoporosis is seen commonly among individuals of older age, who also comprise the predominant group of patients requiring dental implant rehabilitation to enhance the retention of a full denture. Several studies using experimental animal models have demonstrated that an induced osteoporotic condition, before or during implant installation, can negatively affect the osseointegration process and significantly reduce bone-to-implant contact [12–14]. Thus, it is indispensable to fully elucidate the underlying biological mechanisms of altered bone metabolism on osseointegration, from both scientific and clinical perspectives.

The success of implant treatment is, firstly, associated with their early osseointegration and, secondly, their long-term performance on the maintenance of this condition. Although there are several studies involving implant osseointegration in a well-established osteoporotic condition, information about the impact of altered bone metabolism on peri-implant bone tissue after the establishment of osseointegration is scarce. While it is apparent that dental implants placed in osteoporotic patients have a risk of failure of osseointegration, it can be hypothesized that such an alteration of bone metabolism developed after dental implant placement can also negatively affect osseointegration [15]. However, there is no scientific literature reporting on the effect of the distribution of bone metabolism on peri-implant bone characteristics of already-well-integrated bone implants.

The ovariectomized (OVX) rat is one of the most reproducible models to mimic an osteoporotic condition in the trabecular bone and widely used to evaluate the bone–biomaterials response [16]. Therefore, the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) and other international societies recommend this model for research on bone characteristics in response to an osteoporotic condition [17,18]. We here aimed to evaluate, using histological and histomorphometric analysis, whether the induction of an osteoporotic condition, after the acquisition of implant osseointegration, influences peri-implant bone, i.e., bone-to-implant contact (BIC%), and peri-implant bone formation (BA%).

2. Materials and Methods

2.1. Titanium Implants

The implants were produced from pure commercial titanium rods (Aries Alloys, the Netherlands). All implants were cylindrical in shape with a diameter of 2 mm and a length of 4 mm. The surfaces of the implants were grit-blasted (roughness, $R_a = 0.5 \mu\text{m}$) and rinsed ultrasonically for 15 min in the following: 10% nitric acid, 100% acetone and 75% ethanol. Subsequently, all implants were air-dried and sterilized by an autoclave.

2.2. Study Animals

The study was approved by the Animals Ethics Committee at King Saud University, College of Dentistry, Riyadh, Saudi Arabia (4/67/389683). All in vivo experiments followed the ARRIVE guidelines. The study sample comprised a total of 16 healthy female Wistar rats (age ~12 weeks and weighing around ~250 g). The study animals were housed, under veterinary supervision, in standardized rat cages (4–5 animals per cage) and maintained in controlled environmental conditions with regular 12 h light/dark cycles. The animals were fed a standard diet of rat food and water ad libitum.

2.3. Experimental Surgical Protocol

The animals were anesthetized with a single intraperitoneal injection of 0.2 mg/kg xylazine (Chanazine, Chanelle Pharmaceutical, Galway, Ireland) and 0.5 mg/kg ketamine hydrochloride (Ketamine, Pharmazeutische Proparate, Giessen, Germany). Afterward, the right legs (hind limbs) were shaved and decontaminated with an antiseptic iodine solution (Alphadin, MedicScience Life Care Pvt. Ltd., Haryana, India). A longitudinal parapedicular skin incision, 2 cm in length, was made along the midline over the distal femoral condyle. The knee joint capsule was identified through blunt dissection of the skin

flap and incised longitudinally. The patellar ligament was elevated and retracted laterally for complete exposure of the knee joint and distal femoral condyle. Using surgical drills in a low-speed rotary drill (800 rpm), along with saline irrigation as coolant, a cylindrical 2 mm hole paralleling the long axis of the femoral shaft was created in the intercondylar notch, and implants were inserted in the prepared holes (1 implant per rat, Figure 1).

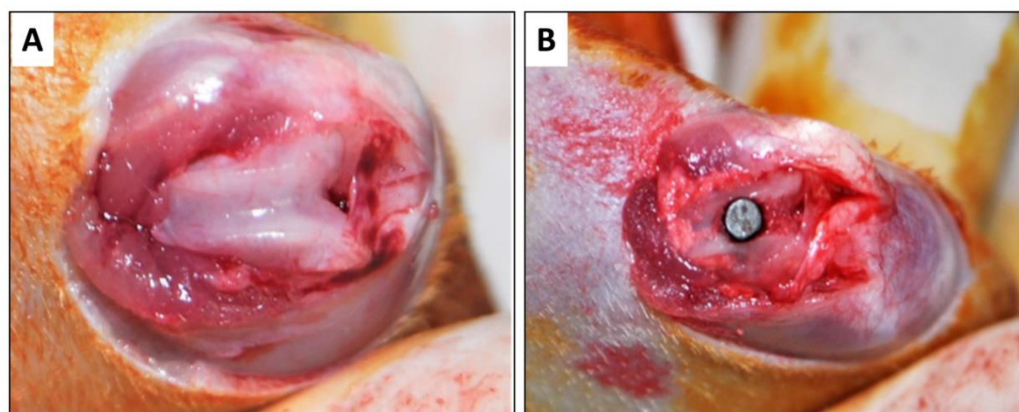


Figure 1. Experimental surgical procedure showing: (A) exposure of the intercondylar notch in the distal femur (knee joint region) and (B) the titanium implant placed within the prepared hole.

Following implantation, the skin and fascia were closed in separate layers with re-sorbable 0.4 sutures (VICRYL[®] Polyglactin 910, Ethicon, Johnson & Johnson, New Brunswick, NJ, USA). Eight weeks following implantation, the rats were randomly subjected to OVX or sham-operated (SHAM) surgeries under GA, as previously described [16]. Six weeks later, OVX and SHAM animals were euthanized. Bone specimens along with implants were harvested and processed for histological and histomorphometric evaluation. The timeline of the experimental design is schematically presented in Figure 2.



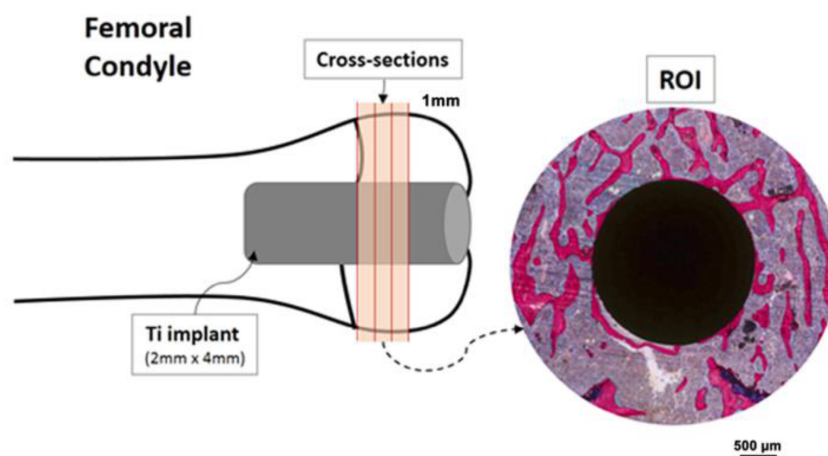
Figure 2. Experimental animal groups and timeline for surgical procedures and sacrifice in the study animals.

2.4. Histological Specimen Preparation and Evaluation

For histological assessment, samples were harvested and fixed in 10% normal buffered formaldehyde. Then, specimens were dehydrated in increasing concentrations of ethyl alcohol from 70% to 100% and subsequently embedded in poly(methylmethacrylate) (pMMA) resin. The pMMA was prepared by a mixture of 600 mL of a methyl methacrylate monomer (Acros Organics BVBA, Geel, Belgium), 60 mL of dibutyl phthalate (Merck KGaA, Darmstadt, Germany) and 1.25 g perkadox (AkzoNobel, Amsterdam, the Netherlands). After polymerization, specimens were cut into slices of approximately 10 μ m thickness and made perpendicularly on the longitudinal axis of the implant using a diamond-coated disc (Leica Microsystems, model SP 1600). The sections were cut starting $\sim$ 1 mm from the top of the implant (Figure 3). Staining for histological analyses of the sections was performed with methylene blue and basic fuchsin.

Table 1. Quantitative histomorphometric variables for the two study groups (presented as mean $\pm$ SD).

	SHAM ($n = 7$)	OVX ($n = 8$)	<i>p</i> -Value
Bone area (BA%)	52.62 $\pm$ 12.68	34.23 $\pm$ 4.31	0.0013
Bone–implant contact (BIC%)	59.02 $\pm$ 19.0	42.5 $\pm$ 20.4	0.0267

**Figure 3.** Illustration for the sectioning procedure. Cross-sections were made starting 1 mm from Table 1. Millimeter circumferential area in which the bone area (BA %) as well as the bone–implant contact (BIC %) were calculated.

Histological images of the sections were obtained using a scanning light microscope (Aperio ImageScope, Leica Biosystems, Buffalo Grove, IL, USA). Histomorphometric analyses of the bone-to-implant contact (BIC) and bone area (BA) percentage were analyzed with the aid of an imaging analysis system software (ImageJ 1.4, National Institute of Health, Bethesda, MD, USA). The histomorphometric measurements were performed for three histological sections per implant (at $\times 20$ objective magnification) and in a blinded manner. The region of interest (ROI) for the bone area (BA%) was defined as a circle including the implant and a 1 mm circumferential area (Figure 3). Bone-to-implant contact (BIC%) was calculated by measurement of the relative length of the implant circumference contacting the bone tissue. The average of these measurements was used for statistical analysis and presented as mean values.

2.5. Statistical Analysis

For the quantitative data, the mean values and standard deviation (SD) were calculated. Statistical analyses were performed using GraphPad InStat (version 3.05, GraphPad Software, San Diego, CA, USA). A Shapiro–Wilk test for normality was conducted on all parameters. The differences in the mean values between the two study groups were evaluated by an unpaired Student's *t*-test. A result was considered significant if $p < 0.05$.

3. Results

3.1. Experimental Animals

All animals remained healthy until the end of the experiment, except one rat (from the SHAM group), which died immediately after implant placement surgery due to GA complications. None of the remaining animals presented any undesirable clinical conditions (e.g., wound infection) or surgical complications during the healing period.

3.2. Qualitative Histological Evaluation of Bone–Implant Interface

In Figure 4, light microscopical images are presented for the OVX and SHAM groups sacrificed after 14 weeks of implantation. The SHAM animals demonstrated a dense

trabecular network. In areas where no bone was formed, some intervening fibrous or marrow tissue was present between the bone trabeculae. Newly formed bone was in close contact with the implant surface, and the implant was observed to be covered for the major part with a thin layer of bone. No intervening fibrous tissue layer was observed between the bone and implant surface. The original drill hole could no longer be recognized. Histological sections of the OVX specimens revealed that the trabecular bone had an osteopenic appearance. The trabecular network was less dense and more irregular compared to that in the SHAM animals. A sparse thin layer of bone formed in direct contact with the implant surface. However, the coverage of the implant surface was less compared to that in the SHAM animals.

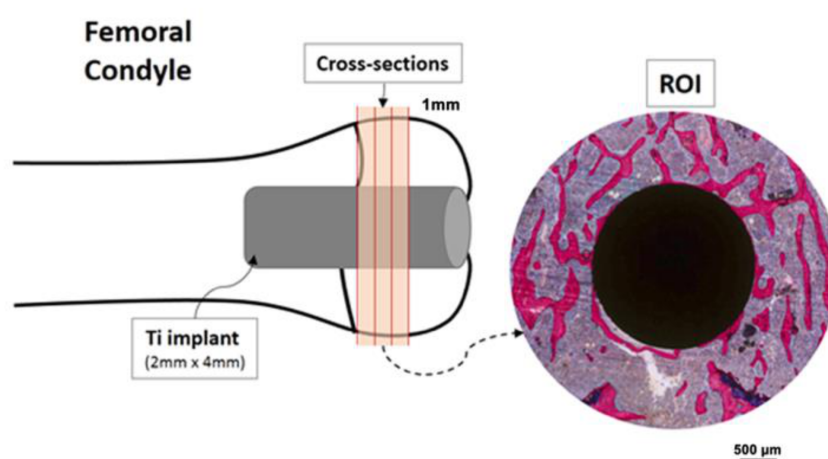


Figure 4. Cross-sectional light microscopical images (left column) show the evident presence of a trabecular bone network around implants in the sham-operated (SHAM) rats. The trabecular network was less dense around the implants in the ovariectomized (OVX) rats. In the identified ROI (right column, magnification 20×), more trabecular bone was observed surrounding and in direct contact with implants in the SHAM rats. In OVX rats, the bone around the implant surface is less, thin and sparse.

In Figure 5, high magnification images show an eroded appearance of the bone trabeculae in the OVX rats. Occasionally, osteoclast-like cells were observed adjacent to the trabeculae near the eroded areas. Furthermore, an apparent higher number of fat cells and a smaller number of plasma cells were present in the bone marrow of the OVX rats compared to that in the SHAM rats. Occasionally, OVX animals showed a fibrous tissue layer intervening between the bone and implant surface.

3.3. Quantitative Histological Evaluation

After 14 weeks of implant healing, histomorphometric analysis revealed that the amount of bone around implants in OVX rats was lower compared to the sham-operated rats (Table 1 and Figure 6). Statistically, BA% in the selected ROI showed a significant reduction in OVX animals compared to the SHAM animals ($p < 0.05$). In addition, the statistical analysis of the bone–implant contact (BIC%) revealed significantly lower values for all implants in OVX versus SHAM animals ($p < 0.05$).

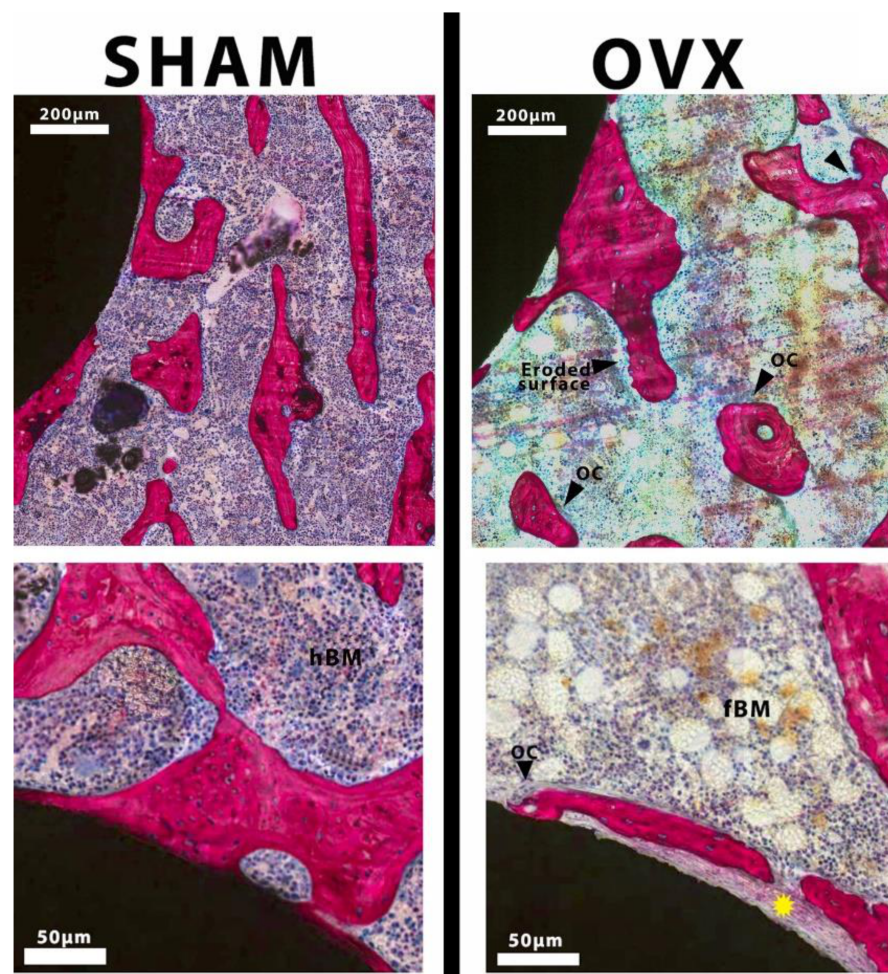


Figure 5. Representative histological images at higher magnification showing eroded bone trabeculae in OVX rats (black arrowhead). Osteoclast (OC)-like cells were present. Bone marrow in the OVX rats contained more fat cells (fBM) in comparison to the hypercellular bone marrow (hBM) in the SHAM group. In some OVX sections, a fibrous tissue layer (yellow star) was detected intervening between bone and implant surface.

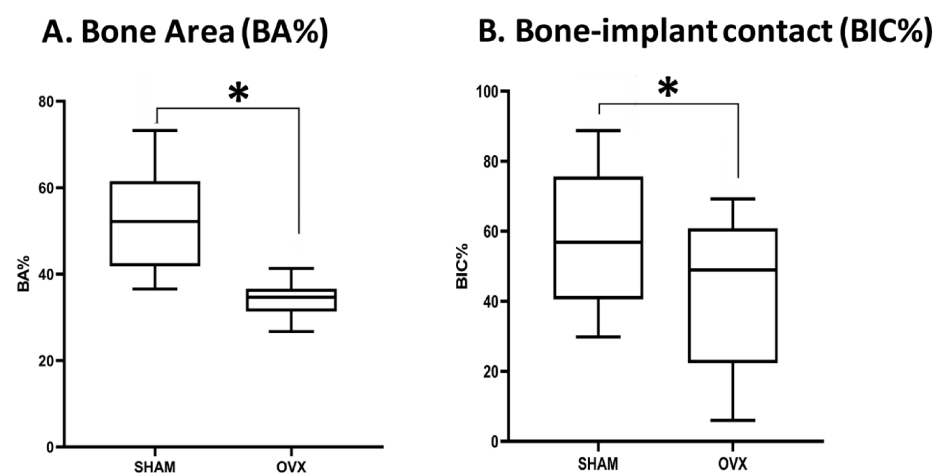


Figure 6. Box-and-whisker plot shows that the histomorphometrical, A, bone area (BA%) and, B, bone-to-implant contact (BIC%) around implants were significantly lower in OVX compared to SHAM rats. (* indicates $p < 0.05$)

4. Discussion

The current *in vivo* experiment intended to test the effect of an OVX-induced osteoporotic condition on the bone tissue surrounding already-osseointegrated implants. Using a 14-week implantation period with osteoporotic induction at 8 weeks, the evaluation of histological images recorded for osteoporotic animals showed an obvious alteration in the quantity and quality of trabecular bone around implants compared to healthy animals. Our results showed that peri-implant bone characteristics in terms of BA% and BIC% were significantly lower in osteoporotic versus healthy animals.

The osseointegration of implants involves a cascade of cellular and extracellular biological events that take place at and around the bone–implant interface [19]. Peri-implant healing is regulated by growth and differentiation factors released by the activated blood cells in the created implant bed, finally reaching an equilibrium status between bone resorption and formation around the implant [20]. This process is initiated within the site of implant osteotomy, immediately after implant placement. The sequence begins with the formation of a blood clot, which organizes and gives rise to woven bone and, finally, leads to lamellar bone formation [21]. Therefore, it is widely believed that bone health and quality are crucial requirements for the success of an implant [22].

A major systemic condition, which adversely affects osseointegration and implant survival, is osteoporosis [23].

Ovariectomized (OVX) rats represent an accepted animal model for simulating osteoporotic conditions [24]. However, histological and radiological studies have presented conflicting results. This can be related to the variation in the age of the animals, different anatomical sites, diverse assessment methods and regions of interest. For instance, previous studies have reported that long bones and the spine are more affected by osteoporosis than jaw bones [25]. In contrast, Hsu et al. showed a loose trabecular bone structure in the femoral necks and mandibles of ovariectomized rats while cortical bone morphology remained unchanged [26].

Moreover, earlier studies have confirmed that trabecular bone characteristics in femoral condyles change within 6–12 weeks after OVX surgery, bearing similarity to osteoporotic changes in postmenopausal women [25,27,28]. An osteoporotic condition was also achieved in the currently used ovariectomized rats, as confirmed by our histological analysis.

In the literature, it has already been postulated that placing implants in a well-established osteoporotic animal model might negatively affect the bone–implant response. For instance, a recent systematic review investigated whether implant osseointegration is compromised in experimental osteoporotic conditions [29]. Despite the large heterogeneity in data, several *in vivo* studies suggested lower osseointegration in osteoporotic animals. This corroborates with Li et al. [30] and Carvalho et al. [31], who also observed lower bone values around implants placed in well-established osteoporotic animals compared to sham-operated rats.

The present study was designed in such a way to replicate the effects of late-induced osteoporosis after the osseointegration of implants was established. At 14 weeks after implantation, the amount of bone around implants was significantly decreased in the osteoporotic rats. This result indicates that the osseointegration of implants may be compromised in an osteoporotic bone condition induced postimplantation.

Our findings corroborate those of Sakakura et al. [32], who used a similar experimental design. After 12-week postimplant placement, the radiographic assessment revealed a significantly low bone density in OVX rats compared to SHAM [32]. However, several differences with the present study exist: (i) Sakakura et al. used the tibia as an implantation site, whereas we placed implants in the femoral condyle, which is composed of trabecular bone and therefore more appropriate to study the bone–implant response [16]. (ii) The data of Sakakura et al. were based on the assessment of the radiographic gray-levels of bone density around implants. It can be argued that the bone density values are suboptimal because this method depends on the used radiographic resolution and gray-scales. In

addition, radiographs represent a 2D representation of a 3D object, which can skew the measurements. In the present study, “gold-standard” histological and histomorphometric analyses were used. Finally, the healing time (i.e., 14 weeks) postimplant placement in the current study was extended compared to the 12-week endpoint in Sakakura et al.’s study.

Although the present findings showed that osteoporotic conditions alter peri-implant bone characteristics after implant osseointegration, the involved mechanism remains unclear. It can be assumed that osteoporosis affects osteoblast/osteoclast proliferation and activity in the vicinity of titanium implants [33]. Specifically, postestrogen deficiency can induce an imbalance in the expression of several molecules and cell receptors (e.g., RANKL and OPG), resulting in peri-implant bone alterations [34]. Although the osteoporotic rat model is a well-established preclinical model for simulating osteoporotic bone conditions and studying peri-implant bone characteristics, bone formation and remodeling in a rat occurs faster than in humans. In addition, rats lack well-developed Haversian systems [35]. As such, differences among species should be considered, and similar studies in large animals are recommended before transferring promising findings to clinical trials with human patients.

Clinically, August et al. [36] previously reported a higher rate of implant loss (13.6%) in the maxilla of osteoporotic women without antiosteoporotic treatment compared with patients with treatment (6.3%, $p = 0.039$). Similarly, Giro et al. [37] performed a systematic review of 12 clinical studies out of 943 potentially eligible articles. They aimed to assess the failure rate of dental implants as placed in osteoporotic patients. Although many osteoporotic patients displayed an increased risk of implant failure, no randomized clinical trial (RCT) was accessible to make a definitive conclusion. Evidently, the clinical literature shows a lack of studies assessing the influence of osteoporotic condition postimplantation.

Furthermore, it has to be emphasized that it is not clear whether the osteoporotic process can be reversed and the amount of bone after osteoporotic therapy will increase again. Therefore, it is recommended that more research is conducted toward this phenomenon considering the still-increasing numbers of dental implants applied to an aging world population. The present in vivo evidence of the impact of an osteoporotic condition on bone behavior around implants supports the need for the fine-tuning of dental implant treatment protocols in compromised clinical situations.

5. Conclusions

We here demonstrate that inducing an osteoporotic condition eight weeks following implant installation in an established rat model negatively affects peri-implant bone characteristics. Further pre(clinical) studies should be considered in order to explore the clinical implications of this finding.

Author Contributions: Conceptualization, A.M.B., M.Y.S., A.A.N., J.J.v.d.B., J.A.J. and H.S.A.; data curation, A.M.B.; formal analysis, M.Y.S.; funding acquisition, H.S.A.; investigation, A.M.B. and M.Y.S.; methodology, A.M.B., M.Y.S., A.A.N., J.J.v.d.B. and H.S.A.; project administration, A.A.N.; resources, A.A.N. and J.J.v.d.B.; validation, J.J.v.d.B. and J.A.J.; visualization, J.A.J.; writing—original draft, A.M.B.; writing—review and editing, J.J.v.d.B., J.A.J. and H.S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deanship of Scientific Research at King Saud University grant number (RG-1439-026).

Institutional Review Board Statement: The present study was approved by the Animal Ethical Committee at King Saud University, College of Dentistry, Riyadh, Saudi Arabia (Approval No. 4/67/389683). All in-vivo experiments obeyed the guidelines (national and international) for animal care and conformed to the ARRIVE guidelines.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Vincent Cuijpers, Martijn Martens and Natasja van Dijk for their help in technical support and assistance in the histological procedure. The authors acknowledge Mary Grace Baysa Vigilla and Anne Rhodanne Lambarte for their support during animal experimental work. Lastly, the authors would like to thank Terrence Sumeng for his assistance during the histomorphometric measurements.

Conflicts of Interest: The authors declare no conflict of interest related to this study.

References

- Gokcen-Rohlig, B.; Yaltirik, M.; Ozer, S.; Tuncer, E.D.; Evlioglu, G. Survival and success of ITI implants and prostheses: Retrospective study of cases with 5-year follow-up. *Eur. J. Dent.* **2009**, *3*, 42. [\[CrossRef\]](#)
- Zupnik, J.; Kim, S.W.; Ravens, D.; Karimbux, N.; Guze, K. Factors associated with dental implant survival: A 4-year retrospective analysis. *J. Periodontol.* **2011**, *82*, 1390–1395. [\[CrossRef\]](#)
- Branemark, P.I.; Hansson, B.O.; Adell, R.; Breine, U.; Lindstrom, J.; Hallen, O.; Ohman, A. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand. J. Plast. Reconstr. Surg. Suppl.* **1977**, *16*, 1–132.
- Puleo, D.A.; Nanci, A. Understanding and controlling the bone-implant interface. *Biomaterials* **1999**, *20*, 2311–2321. [\[CrossRef\]](#)
- Davies, J.E. Bone bonding at natural and biomaterial surfaces. *Biomaterials* **2007**, *28*, 5058–5067. [\[CrossRef\]](#)
- Raikar, S.; Talukdar, P.; Kumari, S.; Panda, S.K.; Oommen, V.M.; Prasad, A. Factors affecting the survival rate of dental implants: A retrospective study. *J. Int. Soc. Prev. Community Dent.* **2017**, *7*, 351. [\[CrossRef\]](#)
- Wirth, A.J.; Goldhahn, J.; Flaig, C.; Arbenz, P.; Müller, R.; van Lenthe, G.H. Implant stability is affected by local bone microstructural quality. *Bone* **2011**, *49*, 473–478. [\[CrossRef\]](#) [\[PubMed\]](#)
- Russell, R.G.G. Bisphosphonates: The first 40 years. *Bone* **2011**, *49*, 2–19. [\[CrossRef\]](#)
- Peck, W. Consensus development conference: Diagnosis, prophylaxis, and treatment of osteoporosis. *Am. J. Med.* **1993**, *94*, 646–650.
- Mellado-Valero, A.; Ferrer-García, J.C.; Calvo-Catalá, J.; Labaig-Rueda, C. Implant treatment in patients with osteoporosis. *Med. Oral Patol. Oral Cir. Bucal.* **2010**, *15*, e52–e57. [\[CrossRef\]](#)
- Friedlander, A.H. The physiology, medical management and oral implications of menopause. *J. Am. Dent. Assoc.* **2002**, *133*, 73–81. [\[CrossRef\]](#) [\[PubMed\]](#)
- Giro, G.; Gonçalves, D.; Sakakura, C.E.; Pereira, R.M.R.; Júnior, E.M.; Orrico, S.R.P. Influence of estrogen deficiency and its treatment with alendronate and estrogen on bone density around osseointegrated implants: Radiographic study in female rats. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol.* **2008**, *105*, 162–167. [\[CrossRef\]](#) [\[PubMed\]](#)
- Slagter, K.W.; Raghoobar, G.M.; Vissink, A. Osteoporosis and edentulous jaws. *Int. J. Prosthodont.* **2008**, *21*, 19–26. [\[PubMed\]](#)
- Tsolaki, I.N.; Madianos, P.N.; Vrotsos, J.A. Outcomes of dental implants in osteoporotic patients. A literature review. *J. Prosthodont.* **2009**, *18*, 309–323. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jeffcoat, M. The association between osteoporosis and oral bone loss. *J. Periodontol.* **2005**, *76*, 2125–2132. [\[CrossRef\]](#)
- Alghamdi, H.S.; van den Beucken, J.J.; Jansen, J.A. Osteoporotic rat models for evaluation of osseointegration of bone implants. *Tissue Eng. Part C Methods* **2014**, *20*, 493–505. [\[CrossRef\]](#)
- Langhof, H.; Chin, W.W.L.; Wieschowski, S.; Federico, C.; Kimmelman, J.; Strech, D. Preclinical efficacy in therapeutic area guidelines from the US Food and Drug Administration and the European Medicines Agency: A cross-sectional study. *Br. J. Pharmacol.* **2018**, *175*, 4229–4238. [\[CrossRef\]](#)
- Tarantino, U.; Iolascon, G.; Cianferotti, L.; Masi, L.; Marcucci, G.; Giusti, F.; Marini, F.; Parri, S.; Feola, M.; Rao, C. Clinical guidelines for the prevention and treatment of osteoporosis: Summary statements and recommendations from the Italian Society for Orthopaedics and Traumatology. *J. Orthop. Traumatol.* **2017**, *18*, 3–36. [\[CrossRef\]](#)
- Fini, M.; Giavaresi, G.; Torricelli, P.; Borsari, V.; Giardino, R.; Nicolini, A.; Carpi, A. Osteoporosis and biomaterial osteointegration. *Biomed Pharm.* **2004**, *58*, 487–493. [\[CrossRef\]](#)
- Davies, J. Mechanisms of endosseous integration. *Int. J. Prosthodont.* **1998**, *11*, 391–401.
- Ballantyne, E. Bisphosphonates: Possible modes of action and implications for dental implant treatment. A review of the literature. *J. Gen. Pract.* **2015**, *3*, 2. [\[CrossRef\]](#)
- Pal, H.; Nair, K.C.; Sinha, S. Bone implant contact and its relationship with strain in the surrounding bone. *J. Interdiscip. Dent.* **2018**, *8*, 102. [\[CrossRef\]](#)
- De Medeiros, F.; Kudo, G.; Leme, B.; Saraiva, P.; Verri, F.; Honório, H.M.; Pellizzer, E.; Junior, J.S. Dental implants in patients with osteoporosis: A systematic review with meta-analysis. *Int. J. Oral Maxillofac. Surg.* **2018**, *47*, 480–491. [\[CrossRef\]](#) [\[PubMed\]](#)
- Calciolari, E.; Donos, N.; Mardas, N. Osteoporotic animal models of bone healing: Advantages and pitfalls. *J. Investig. Surg.* **2017**, *30*, 342–350. [\[CrossRef\]](#)
- Lee, C.; Lee, J.-H.; Han, S.-S.; Kim, Y.H.; Choi, Y.-J.; Jeon, K.J.; Jung, H.I. Site-specific and time-course changes of postmenopausal osteoporosis in rat mandible: Comparative study with femur. *Sci. Rep.* **2019**, *9*, 1–9. [\[CrossRef\]](#)
- Hsu, P.-Y.; Tsai, M.-T.; Wang, S.-P.; Chen, Y.-J.; Wu, J.; Hsu, J.-T. Cortical bone morphological and trabecular bone microarchitectural changes in the mandible and femoral neck of ovariectomized rats. *PLoS ONE* **2016**, *11*, e0154367. [\[CrossRef\]](#)
- Wronski, T.; Cintron, M.; Dann, L. Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. *Calcif. Tissue Int.* **1988**, *43*, 179–183. [\[CrossRef\]](#)

-
28. Johnston, B.D.; Ward, W.E. The ovariectomized rat as a model for studying alveolar bone loss in postmenopausal women. *Biomed Res. Int.* **2015**, *2015*, 635023. [[CrossRef](#)]
 29. Dereka, X.; Calciolari, E.; Donos, N.; Mardas, N. Osseointegration in osteoporotic-like condition: A systematic review of preclinical studies. *J. Periodontal Res.* **2018**, *53*, 933–940. [[CrossRef](#)]
 30. Li, Y.; He, S.; Hua, Y.; Hu, J. Effect of osteoporosis on fixation of osseointegrated implants in rats. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2017**, *105*, 2426–2432. [[CrossRef](#)]
 31. Carvalho, M.D.; Benatti, B.B.; César-Neto, J.B.; Nociti, F.H., Jr.; da Rocha Nogueira Filho, G.; Casati, M.Z.; Sallum, E.A. Effect of cigarette smoke inhalation and estrogen deficiency on bone healing around titanium implants: A histometric study in rats. *J. Periodontol.* **2006**, *77*, 599–605. [[CrossRef](#)]
 32. Sakakura, C.E.; Giro, G.; Gonçalves, D.; Pereira, R.M.R.; Orrico, S.R.P.; Marcantonio, E., Jr. Radiographic assessment of bone density around integrated titanium implants after ovariectomy in rats. *Clin. Oral Implant. Res.* **2006**, *17*, 134–138. [[CrossRef](#)]
 33. Marco, F.; Milena, F.; Gianluca, G.; Vittoria, O. Peri-implant osteogenesis in health and osteoporosis. *Micron* **2005**, *36*, 630–644. [[CrossRef](#)] [[PubMed](#)]
 34. Tera, T.D.M.; Prado, R.F.D.; de Marco, A.C.; Santamaria, M.P.; Jardini, M.A.N. The RANK/RANKL/OPG interaction in the repair of autogenous bone grafts in female rats with estrogen deficiency. *Braz. Oral Res.* **2014**, *28*, 1–9. [[CrossRef](#)]
 35. Turner, A.S. Animal models of osteoporosis—Necessity and limitations. *Eur. Cell Mater.* **2001**, *1*, 13.
 36. August, M.; Chung, K.; Chang, Y.; Glowacki, J. Influence of estrogen status on endosseous implant osseointegration. *J. Oral Maxillofac. Surg.* **2001**, *59*, 1285–1289. [[CrossRef](#)]
 37. Giro, G.; Chambrone, L.; Goldstein, A.; Rodrigues, J.A.; Zenóbio, E.; Feres, M.; Figueiredo, L.C.; Cassoni, A.; Shibli, J.A. Impact of osteoporosis in dental implants: A systematic review. *World J. Orthop.* **2015**, *6*, 311. [[CrossRef](#)] [[PubMed](#)]