nification). First, a circular region of interest (ROI) with a 3-mm diameter equal to the created defect was identified (Figure 3). Then, within this ROI, the amount of new bone formation (N-BF%), residual graft material (RBG%) and trabecular bone space (Tb.Sp%) were determined as area percentages.

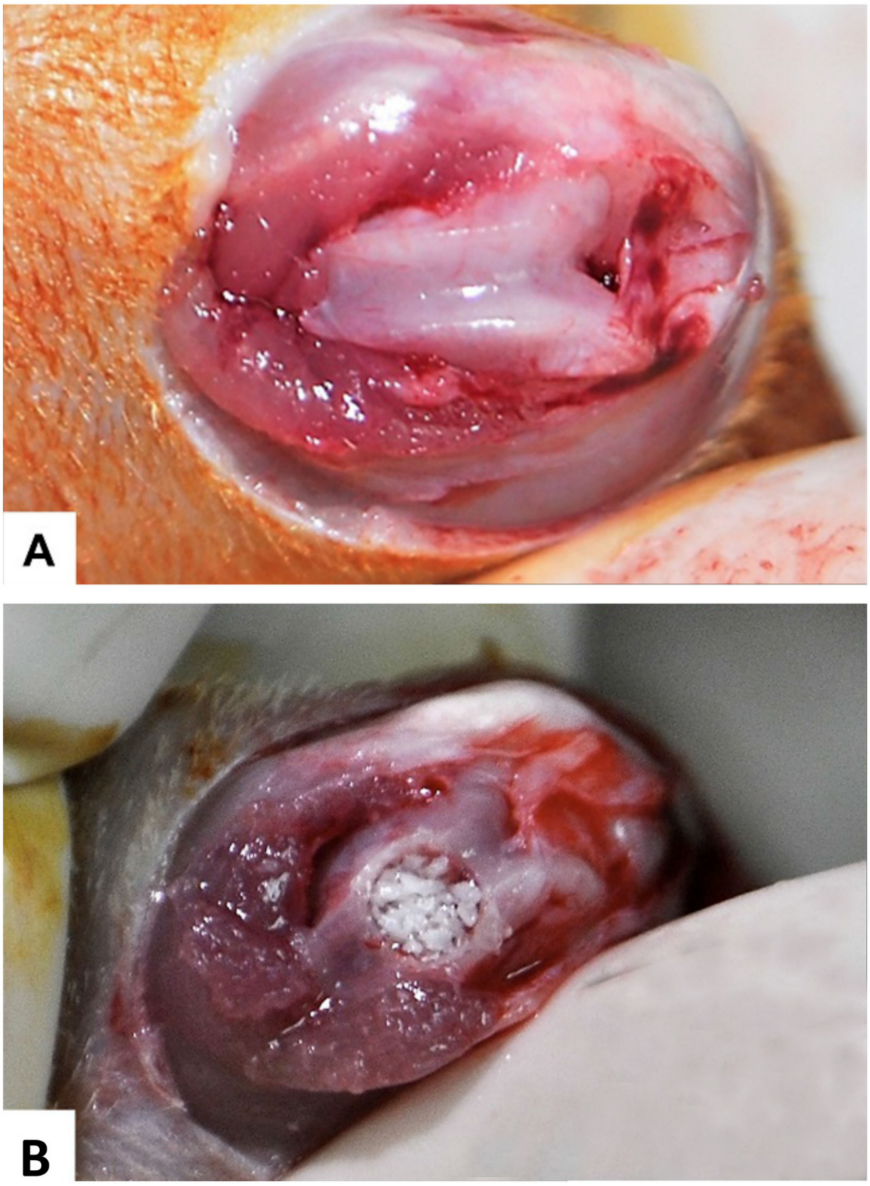

Figure 1. Pictures of the surgical procedure: (**A**) the femoral condyle exposed; and (**B**) bone defect filled with InterOss® material.

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

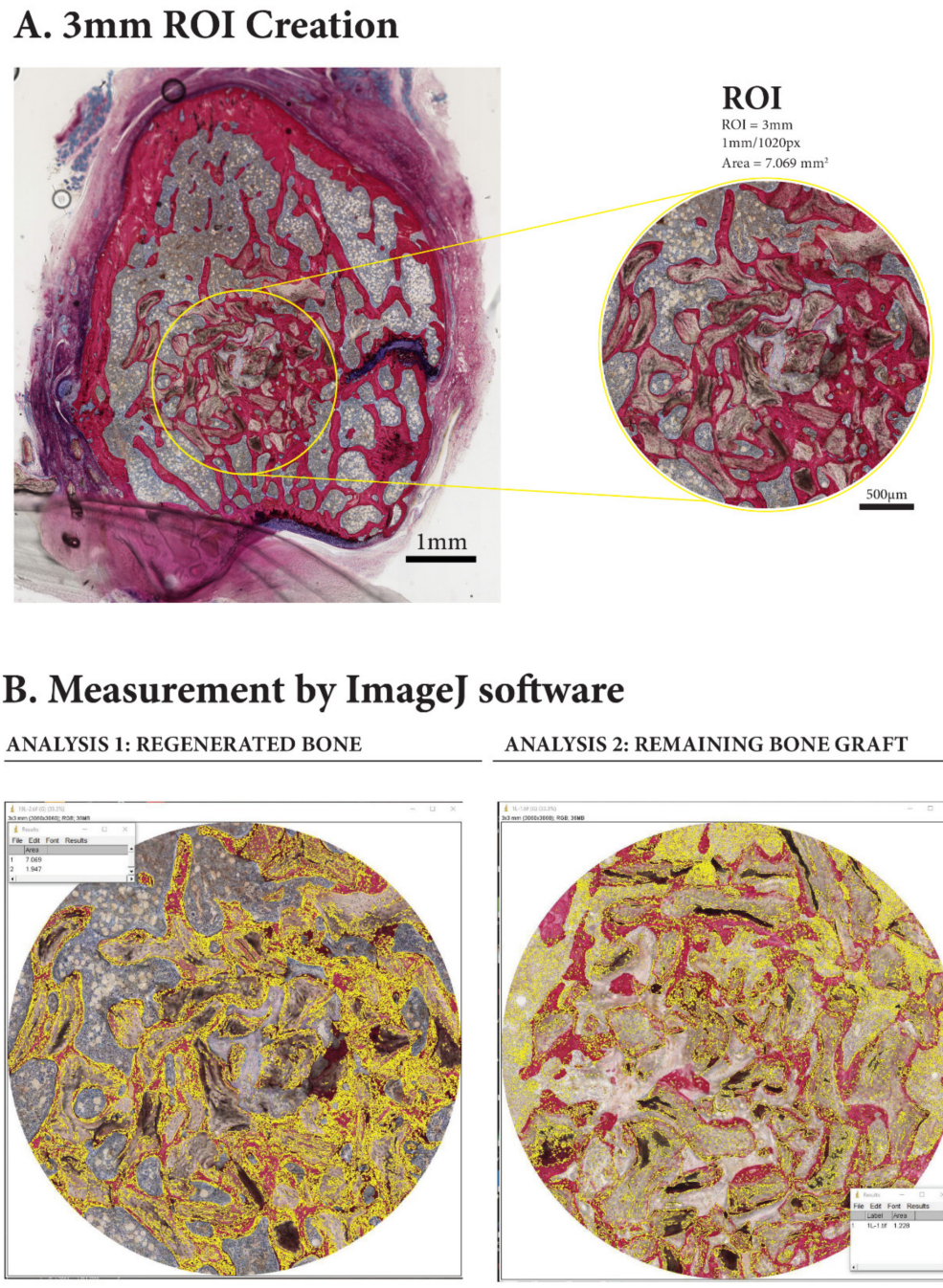

Figure 3. In the histological sections: (**A**) a 3-mm region of interest (ROI) was identified; and (**B**) quantitative measurements were then made to assess new bone formation (N-BF%), remaining bone graft (RBG%), and trabecular bone space (Tb.Sp%) using ImageJ software.

2.5. Statistical Analysis

All quantitative data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using InStat Statistical Program (Version 3.05, GraphPad Software, San Diego, CA, USA). An unpaired Student's *t*-test was conducted to evaluate differences in the mean values between the two study groups. The level of significance was set at 95% ($p < 0.05$).

3. Results

3.1. Animal Observations

Postoperative healing was uneventful in all animals and no complications were observed after the bone grafting and ovariectomy surgeries, except one rat (from SHAM group) that died due to GA complications. Consequently, seven rats in the SHAM group and eight rats in the OVX group were finally examined.

3.2. Descriptive Histological Evaluation

Representative images of the histological sections are depicted in Figure 4. The histological sections of the OVX specimens revealed that the trabecular bone had an osteopenic appearance. The trabecular network was less dense and irregular compared to SHAM sections. At 14 weeks, images showed a higher trabecular number and less intertrabecular spacing for SHAM compared to OVX bone specimens. In between the bone trabeculae, bone marrow-like tissue was observed, characterized by the presence of mononuclear cells. As the bone tissue was stained pink, it could easily be discerned from the grafted InterOss® granules. More granules seemed to remain in the defects in the SHAM animals compared to OVX animals.

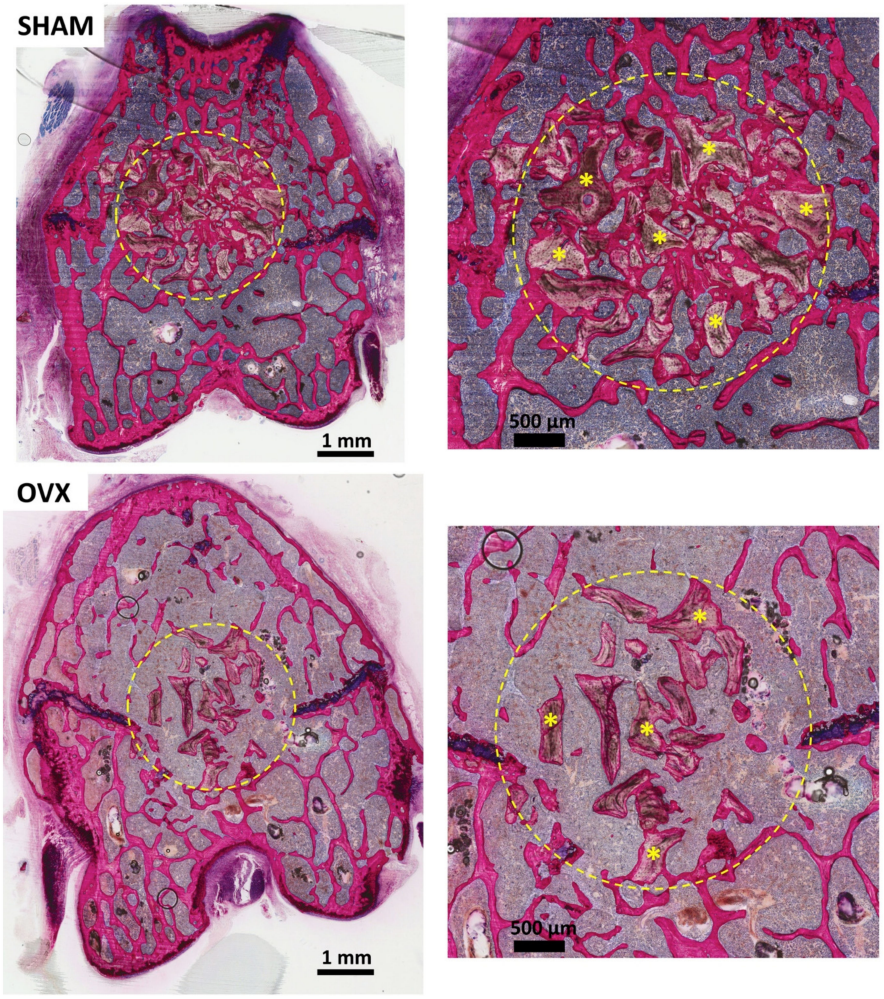

Figure 4. Representative histological images of pMMA sections: (**left**) for SHAM and OVX at 14 weeks post-implantation; and corresponding images at ×20 magnification (**right**). Methylene blue and basic fuchsin staining was performed. Within the ROI, images show the evident presence of new bone, remaining InterOss® material (yellow*) and trabecular bone space (Tb.Sp).

Higher magnification (Figure 5) revealed that the majority of the InterOss® granules in the SHAM specimens were surrounded by abundant new bone formation, which was in direct contact with the graft material. Further, bone bridging was observed between InterOss® granules. The grafted granules seemed to be completely covered by newly formed bone. High magnification images of the OVX specimen showed less newly formed bone in the osseous defects. The bone defect was for the major part filled with bone marrow-like tissue. The bone marrow-like tissue in the OVX rats contained more fat cells and less plasma cells compared to SHAM rats. Frequently, only a very superficial layer of bone was present on the surface of the granules. Occasionally, even no bone at all was seen covering the granules. Further, light micrographs showed the frequent presence of osteoclast-like cells at the interface between bone marrow-like tissue and granules (Figure 5). The images demonstrated also that some bone trabeculae in the OVX rats had an eroded appearance, which could be associated with the presence of osteoclast-like cells.

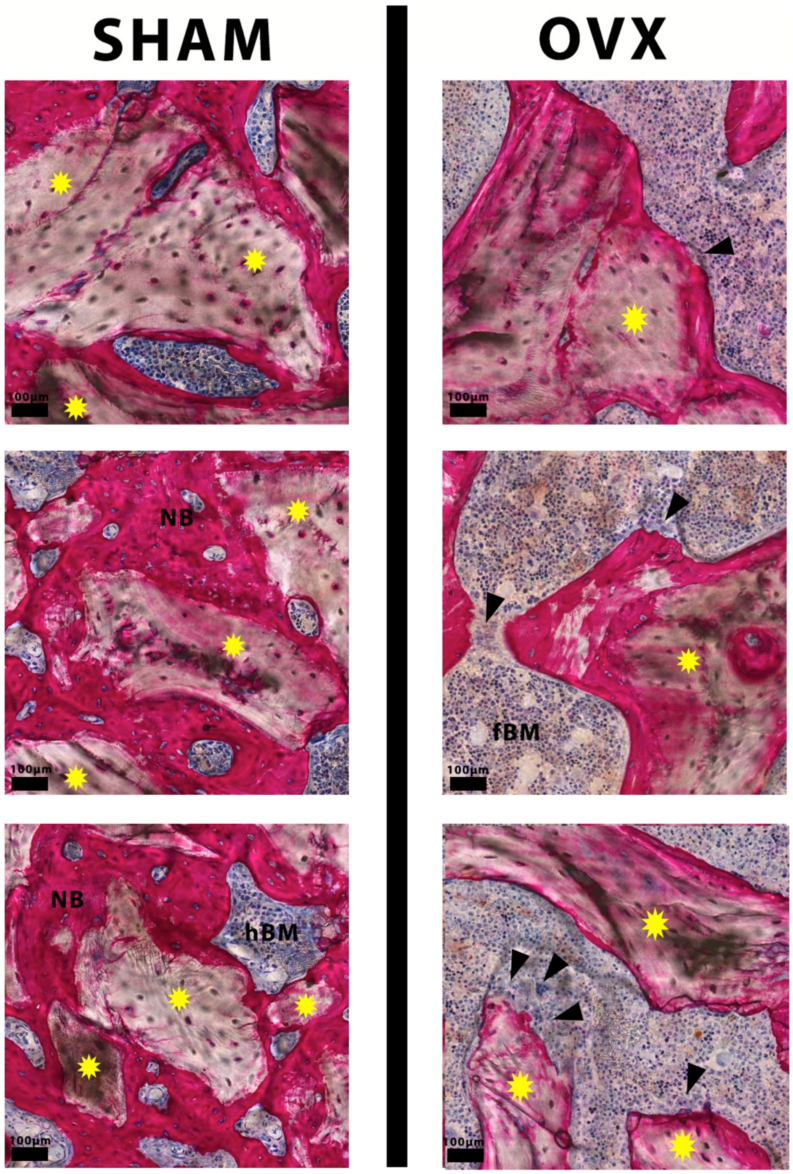

Figure 5. Representative histological images at higher magnification showing InterOss® granules (yellow stars) well integrated and completely covered with newly formed bone (NB) in SHAM rats (**left**). In OVX rats, eroded bone surface and osteoclast-like cells (black arrowheads) were present (**right**). Bone marrow-like tissue in OVX rats contained more fat cells (fBM) compared to the hypercellular bone marrow-like tissue (hBM) in the SHAM group.

3.3. Histomorphometric Evaluation

The results of the histomorphometric evaluation of osseous defects grafted with InterOss® granules are depicted in Table 2 and Figure 6. Data show a significantly decreased amount of new bone formation (N-BF%) in OVX rats compared to SHAM rats ($p < 0.05$). Additionally, the amount of remaining graft material (RBG%) was significantly lower in OVX compared to SHAM ($p < 0.05$). Finally, the mean of trabecular bone space (Tb.Sp%) was significantly higher in OVX compared to SHAM ($p < 0.05$).

Table 2. Quantitative histomorphometric data showing mean ± SD values for new bone formation (N-BF%), remaining bone graft (RBG%) and trabecular bone space (Tb.Sp%) in the two study groups.

	SHAM (n = 7)	OVX (n = 8)
New bone formation (N-BF%)	37.7 ± 7.9 *	22.5 ± 3.0
Remaining bone graft (RBG%)	34.8 ± 9.6 *	23.7 ± 5.8
Trabecular bone space (Tb.Sp%)	27.5 ± 14.3 *	53.8 ± 7.7

* indicates $p < 0.05$.

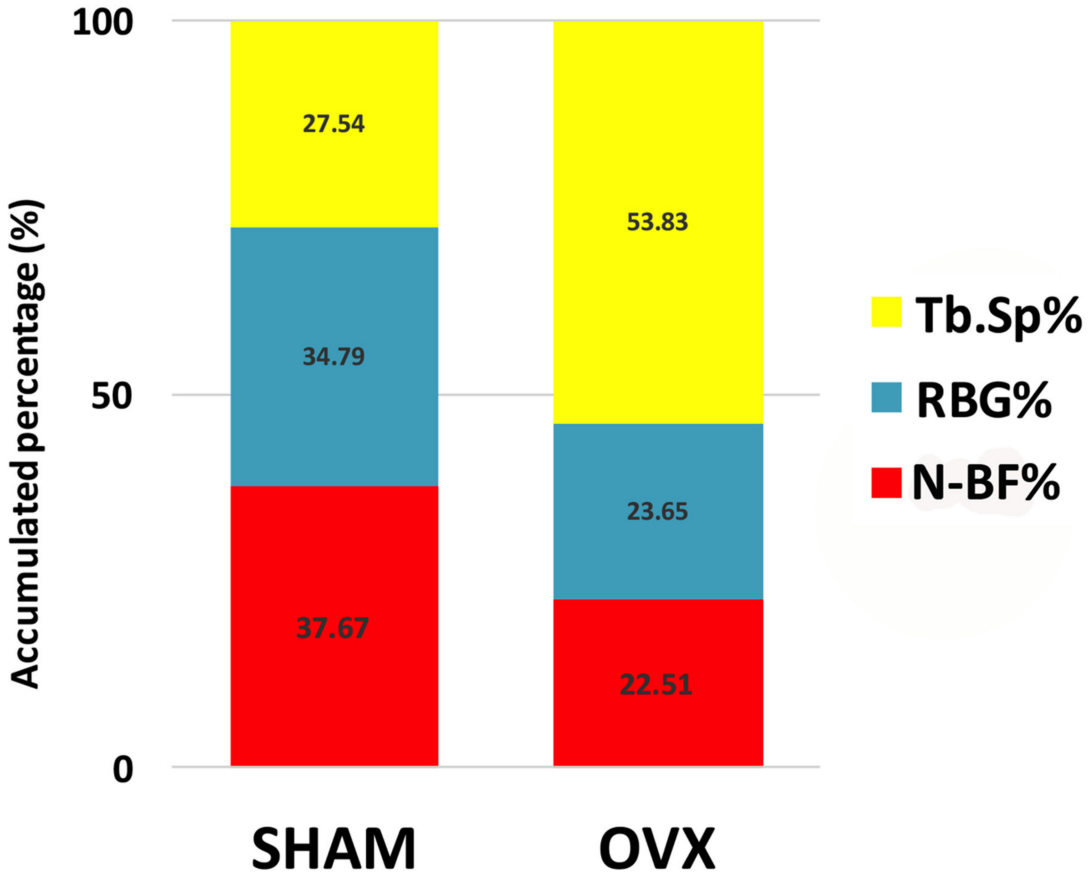

Figure 6. Histomorphometric evaluation of the mean volume fractions of new bone formation (N-BF%, red bar), remaining grafting material (RBG%, blue bar) and trabecular bone space (Tb.Sp%, yellow bar) occupying the defects after the 14 weeks of healing. Statistical analysis showed significant difference between SHAM and OVX for all parameters ($p < 0.05$).

4. Discussion

The present study aimed to evaluate the effect of inducing an osteoporotic condition after eight weeks of initial healing of bone defects grafted with xenograft in a rat model. The results reveal greater new bone formation within osseous defects in healthy compared to osteoporotic bone conditions. On the other hand, reduction in the remaining graft

material was greater in the osteoporotic bone condition. In addition, osteoporotic bone showed larger areas of soft tissue/marrow space compared to healthy bone.

Clinically, deproteinized bovine bone graft material is extensively used to repair osseous defects [8]. Multiple human clinical studies have already been performed and long-term data regarding the outcome of bone grafting procedures have been reported. For instance, Piattelli et al. [7] conducted a histological analysis in 20 patients treated with Bio-Oss up to four years of bone healing. They concluded that anorganic bovine bone is osteoconductive and promotes the successful long-term outcome of bone grafting. In another study by Scarano et al. [25], bovine porous bone mineral for maxillary sinus augmentation was used and then dental implants installed. Histological results in an implant retrieved four years after insertion show direct contact between bone and implant without an interposition of the graft material particles. They concluded that that the slow resorption of the bovine graft particles did not jeopardize the osseointegration of the implant. Further, the results from a six-year randomized-controlled clinical trial with bovine bone material by Stavropoulos and Karring [26] show improvements in intrabony defect healing using radiographs and clinical assessment parameters.

Experiments performed with animal species such as rabbits, goats, sheep, dogs and rodents are often used to study bone regeneration. For mimicking osteoporotic bone conditions, rats were utilized in the present study to simulate alterations in bone regeneration following induced estrogen deficiency as in human. Although rats are small experimental animals and have a different bone formation and remodeling rate compared with humans, they are excellent preclinical models for studying osteoporotic changes as they closely emulate pharmaco-therapeutic response and allow studying the effect of estrogen depletion on the skeleton [27]. However, it has to be noticed that the preclinical model as used in the present study encounters several limitations that need to be solved in prospective studies. For instance, it has been noticed that ovariectomized rats have a faster bone turnover than patients with osteoporosis. Therefore, further in vivo studies can be suggested to validate the effect of osteoporosis on bone regeneration related to different bone substitutes. In addition, a larger animal model should be used to clarify the effect of osteoporosis with bone graft under challenged bone condition.

In animal models of experimental osteoporosis, the assessment of the biomaterial-mediated bone regeneration process in subcritical sized defects, considered established approaches relied on [28]. Despite the proven capability of the reported models, the usage of critical size defects, i.e., intraosseously established wounds that do not report spontaneous healing, has been considered the standard approach for the validation of translational bone regenerative strategies and output the intrinsic regenerative potential of the grafted bone. In the current study, the femoral condyle OVX rat model allowed examined bone regeneration in a 3-mm critical size defect. For instance, we recently published an in vivo experimental study using the same femoral condyle model of 3-mm critical size defect. The untreated (empty) defects did not heal without intervention, making the rat femoral condyle model a well-established and standardized critical size defect model highly useful for evaluating bone regeneration in healthy and osteoporotic bone [29].

The biological performance of deproteinized bovine bone grafts is widely investigated in preclinical studies using healthy animals [24,30–34]. These confirmed that bovine bone particles enhance bone regeneration, and its remnants can become integrated very well with the newly formed bone. In many histological studies, osteoblasts and osteoclasts were observed in conjunction with bovine bone particles as well as with the newly formed bone. Intimate contact between the implanted material and the newly formed bone was also commonly presented. For instance, van Houdt et al. [24] tested bovine bone (Bio-Oss) implanted in femoral condylar bone defects in rats. At 12 weeks, new bone formation in direct contact with the Bio-Oss granules was observed. The remaining Bio-Oss granules were completely covered by new bone. In line with those observations, histological analysis in the present study demonstrated similar findings in the SHAM (healthy) animals using InterOss® bone granules after 14 weeks of healing (Figure 5).

Beside the grafted material, the bone condition is the key factor affecting the graft healing and its integration with new bone [16,35,36]. In many clinical cases, the presence of osteoporosis is a major challenging condition in patients undergoing bone grafting surgery due to decreased capacity of bone regeneration [16,37]. In a retrospective analysis of 49 patients, an alveolar bone grafting procedure was impaired in 11 patients due to an unfavorable bone condition (i.e., osteopenia) [37]. This is primarily due to estrogen deficiency, which negatively influences the bone metabolism [38]. Estrogen deficiency causes an imbalance in osteoblastic/osteoclastic processes and results in an increased breakdown of bone and a reduced bone formation [39].

In previous preclinical studies, ovariectomized rats were used to evaluate qualitatively and quantitatively the influence of estrogen deficiency on bone grafting [40,41]. For example, Luize et al. [41] examined bone blocks to augment a mandibular defect in OVX rats. At 7, 14 and 28 days post-surgery, histological analysis showed a delay in the osteogenic activity and bone healing in OVX rats compared to SHAM rats. The majority of grafted materials in OVX animals appeared to be interspaced by fibrous tissue. Additionally, OVX rats exhibited significantly less new bone formation compared to SHAM rats. This result is in accordance with the present study.

In contrast, some other animal studies did not observe a significant effect in terms of new bone formation related to bone grafting in osteoporotic versus healthy animals [24,42–44]. This discrepancy might be due to differences in the experimental design, animal model, type of biomaterials, bone defects and evaluation periods. Therefore, it is difficult to establish direct correlations between these studies.

Despite the contradiction in previous studies, the present findings sustain an impaired regenerative potential of bone grafting in OVX animals. Differently, our study model was designed in such a way to replicate the effects of late-induced osteoporosis after the bone grafting procedure was performed. At 14 weeks of bone healing, the quality and quantity of bone formation (N-BF%) was significantly decreased in the osteoporotic rats, which indicates that bone formation in a bone defect can become compromised in an osteoporotic condition, as induced post-implantation. Nevertheless, the exact involved mechanism needs to be explored further in follow-up studies.

Previously, it has been reported that a deficiency in estrogen receptor (i.e., ER-α and ER-β) expression contributes negatively to the regenerative potential in osteoporotic bone [45]. Likewise, osteoblast-related gene expression (e.g., RUNX2, BMP2, COLLAGEN I and OSTEOCALCIN) were significantly decreased in OVX animals in relation to bone-biomaterial regeneration [30,46]. Further, the osteogenic differentiation of mesenchymal stem cells (MSCs) derived from osteoporotic bone was significantly altered [18]. This emphasizes the effect of intrinsic deficiencies in the regenerative capability of osteoporotic bone, particularly in the presence of bone biomaterials. Previous data also verify an increase of the adipogenic activation in the bone of OVX animals [46]. This seems to disrupt the normal osteogenic function within the bone tissue [26]. In agreement, we noticed more fat bone marrow with an hypocellular appearance in OVX rats compared to healthy control rats.

It is important to note that in the current study the amount of remaining graft material was significantly less in the osteoporotic bone conditions compared to healthy bone conditions. We suppose that this is caused by the observed increased presence of osteoclast-like cells around the graft granules in the OVX animals. In addition, we assume that the eroded surfaces of trabecular bone indicate hyperactivity of osteoclastic cells in osteoporotic bone compared to healthy bone. Usually, the remodeling of bone graft material occurs in multiple phases [46]. Bone graft resorption is initiated by the function of osteoclastic cells. Osteoclast activation is directly linked to osteoblast function [35]. The role of osteoclasts is not limited to the resorption of bone. They also have a significant impact on the local recruitment and bone-forming activity of osteoblasts via so-called cell–cell "crosstalk". Bone formation and resorption during remodeling have to be aligned carefully in a process that is called "coupling" [47]. Therefore, an imbalance of osteoclast/osteoblast activities in

osteoporotic bone is connected with the decreased bone formation and increased rate of resorption, which limits the potential of bone-biomaterial incorporation, as observed in our study.

The performance of bone-biomaterials for bone defect augmentation showed limitation, in regard fast resorption rate, which correlated to high number osteoclast (resorbing) cells. One possible way to overcome the mentioned limitation incorporates biomaterial that showed lower number and activity of osteoclast cells. For instance, Westhauser et al. [48] showed that addition 45S5 bioactive glass to β-tricalcium phosphate might be a way to overcome the individual limitations of both materials by a combination of their respective strengths. They concluded that volume of combined materials during the 10-week implantation period remained almost unchanged correlating with significantly decreased physical presence and less pronounced genetic activity of bone-resorbing cell populations (TRAP+). Furthermore, the presence and activity of osteoclasts is highly dependent on the chemical composition of the bone substitute material and also influences osteoblast–osteoclast crosstalk and coupling [47]. The activity of osteoclastic bone resorption can be visualized by specific staining, i.e., tartrate-resistant acid phosphatase (TRAP) staining [30,49]. Decalcification of bone specimens is required for the application of TRAP staining. TRAP staining cannot be performed using pMMA embedding. However, in our study, we were limited to examining the activity of osteoclastic cells via their TRAP expression due to low number of available specimens to prepare decalcified sections. Consequently, osteoclasts could only be analyzed by histological appearance (i.e., relatively larger and multinucleated) at higher magnification (Figure 5). In addition, proper quantification of the number of osteoclasts was not feasible. This has to be considered as a limitation of our study design.

Although the OVX rat model is a well-established preclinical model for simulating osteoporotic bone conditions, differences among species and animal models size should be considered before transferring promising findings to clinical trials with human patients. Small animal models offer the easiest way to keep animals at low costs for longer time with a high reproduction rate. The offer of markers for laboratory use is the biggest in the market. On the other hand, clinical situation is far away. The results must be interpreted very carefully and often require further studies in larger animal models. Large animal models are as close to the clinical situation as a model can be. Organs, blood supply and common physiology are relatively close to humans. Disadvantages are high costs, high personal and work effort, limitation of follow-up period and availability of markers to study special histological issues [50].

In view of the above mentioned, it is very relevant to take care of the potential implication of impaired bone regeneration in osteoporotic conditions in relation to bone grafting procedures. In these situations, a solution can be the development of bone graft material that prospectively promotes the bone healing outcome.

5. Conclusions

Within the limitations of this study, inducing an osteoporotic condition in rats after initial healing of a bone graft material negatively influences bone regeneration in the created bone defect. Further investigations are needed to explore the clinical implications of these findings.

6. Impact Statement

Recently, increasing concerns have existed about the effect of bone diseases, such as osteoporosis, on bone regeneration. However, bone-grafting complications may occur in patients with compromised medical bone condition, e.g. osteoporosis, as bone healing in such patients can be challenged or impaired. This study assessed if altered bone metabolism due to osteoporosis affects bone regeneration related to bone-defect grafting using preclinical animal models. The results suggest that inducing an osteoporotic condition in rats after initial healing of a bone graft material negatively influences bone regeneration in the created bone defect.

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

Funding: The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No (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 are grateful to the technical support of Vincent Cuijpers, Martijn Martens and Natasja van Dijk in histological preparation and sectioning. The authors are also grateful to Mary Grace Baysa Vigilla and Rhodanne Nicole Lambarte for their support during animal experimental work. Finally, the authors thank Terrence Sumague for his assistance during the histomorphometric measurements.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Brunsvold, M.A.; Mellonig, J.T. Bone grafts and periodontal regeneration. *Periodontol. 2000* **1993**, *1*, 80–91. [CrossRef] [PubMed]
2. Nasr, H.F.; Aichelmann-Reidy, M.E.; Yukna, R.A. Bone and bone substitutes. *Periodontol. 2000* **1999**, *19*, 74–86. [CrossRef] [PubMed]
3. Oryan, A.; Alidadi, S.; Moshiri, A.; Maffulli, N. Bone regenerative medicine: Classic options, novel strategies, and future directions. *J. Orthop. Surg. Res.* **2014**, *9*, 18. [CrossRef] [PubMed]
4. Cypher, T.J.; Grossman, J.P. Biological principles of bone graft healing. *J. Foot Ankle Surg.* **1996**, *35*, 413–417. [CrossRef]
5. Goulet, J.A.; Senunas, L.E.; DeSilva, G.L.; Greenfield, M.L. Autogenous iliac crest bone graft. Complications and functional assessment. *Clin. Orthop. Relat. Res.* **1997**, *339*, 76–81. [CrossRef]
6. Allegrini, S., Jr.; Koening, B., Jr.; Allegrini, M.R.; Yoshimoto, M.; Gedrange, T.; Fanghaenel, J.; Lipski, M. Alveolar ridge sockets preservation with bone grafting-review. *Ann. Acad. Med. Stetin* **2008**, *54*, 70–81.
7. Piattelli, M.; Favero, G.A.; Scarano, A.; Orsini, G.; Piattelli, A. Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: A histologic long-term report of 20 cases in humans. *Int. J. Oral. Maxillofac Implant.* **1999**, *14*, 835–840.
8. Gazdag, A.R.; Lane, J.M.; Glaser, D.; Forster, R.A. Alternatives to autogenous bone graft: Efficacy and indications. *Jaaos-J. Am. Acad. Orthop. Surg.* **1995**, *3*, 1–8. [CrossRef]
9. Masaki, C.; Nakamoto, T.; Mukaibo, T.; Kondo, Y.; Hosokawa, R. Strategies for alveolar ridge reconstruction and preservation for implant therapy. *J. Prosthodont. Res.* **2015**, *59*, 220–228. [CrossRef]
10. Khojasteh, A.; Soheilifar, S.; Mohajerani, H.; Nowzari, H. The effectiveness of barrier membranes on bone regeneration in localized bony defects: A systematic review. *Int. J. Oral. Maxillofac. Implant.* **2013**, *28*, 1076–7089. [CrossRef]
11. Esposito, M.; Grusovin, M.G.; Felice, P.; Karatzopoulos, G.; Worthington, H.V.; Coulthard, P. The efficacy of horizontal and vertical bone augmentation procedures for dental implants-a Cochrane systematic review. *Eur. J. Oral Implant.* **2009**, *2*, 167–184.
12. Aytekin, M.; Arisan, V. Alveolar Ridge Augmentation Techniques in Implant Dentistry. In *Oral and Maxillofacial Surgery*; IntechOpen: London, UK, 2020.
13. Cicciù, M.; Fiorillo, L.; Cervino, G.; Habal, M.B. BMP Application as Grafting Materials for Bone Regeneration in the Craniofacial Surgery: Current Application and Future Directions by an RCT Analysis. *J. Craniofacial Surg.* **2020**. [CrossRef] [PubMed]
14. Dervis, E. Oral implications of osteoporosis. *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod.* **2005**, *100*, 349–356. [CrossRef] [PubMed]
15. Calciolari, E.; Mardas, N.; Dereka, X.; Kostomitsopoulos, N.; Petrie, A.; Donos, N. The effect of experimental osteoporosis on bone regeneration: Part 1, histology findings. *Clin. Oral. Implant. Res.* **2017**, *28*, e101–e110. [CrossRef] [PubMed]
16. Thompson, D.; Simmons, H.; Pirie, C.; Ke, H. FDA Guidelines and animal models for osteoporosis. *Bone* **1995**, *17*, S125–S133. [CrossRef]
17. Jiupeng, D.; Mengchun, Q.; Jianping, L.; Ming, Z.; Jinyuan, L.; Yongqiang, L.; Wei, D. Effect of local treatment with Zoledronate Acid on bone healing of Bio-oss bone graft and osseointegration of dental implants in osteoporotic rats. In Proceedings of the 2011 International Conference on Human Health and Biomedical Engineering, Jilin, China, 19–22 August 2011; pp. 973–976.

18. Benisch, P.; Schilling, T.; Klein-Hitpass, L.; Frey, S.N.P.; Seefried, L.; Raaijmakers, N.; Krug, M.; Regensburger, M.; Zeck, S.; Schinke, T. The transcriptional profile of mesenchymal stem cell populations in primary osteoporosis is distinct and shows overexpression of osteogenic inhibitors. *PLoS ONE* **2012**, *7*, e45142. [CrossRef]
19. Teitelbaum, S.L. Osteoclasts, integrins, and osteoporosis. *J. Bone Miner. Metab.* **2000**, *18*, 344. [CrossRef]
20. Jeffcoat, M. The association between osteoporosis and oral bone loss. *J. Periodontol.* **2005**, *76*, 2125–2132. [CrossRef]
21. 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]
22. Shaheen, M.Y.; Basudan, A.M.; de Vries, R.B.; van den Beucken, J.J.; Jansen, J.A.; Alghamdi, H.S. Bone Regeneration Using Antiosteoporotic Drugs in Adjunction with Bone Grafting: A Meta-Analysis. *Tissue Eng. Part. B Rev.* **2019**, *25*, 500–509. [CrossRef]
23. Vesterinen, H.; Sena, E.; Egan, K.; Hirst, T.; Churolov, L.; Currie, G.; Antonic, A.; Howells, D.; Macleod, M. Meta-analysis of data from animal studies: A practical guide. *J. Neurosci. Methods* **2014**, *221*, 92–102. [CrossRef] [PubMed]
24. Van Houdt, C.I.A.; Ulrich, D.J.O.; Jansen, J.A.; van den Beucken, J. The performance of CPC/PLGA and Bio-Oss® for bone regeneration in healthy and osteoporotic rats. *J. Biomed. Mater. Res. B Appl. Biomater.* **2018**, *106*, 131–142. [CrossRef] [PubMed]
25. Scarano, A.; Pecora, G.; Piattelli, M.; Piattelli, A. Osseointegration in a sinus augmented with bovine porous bone mineral: Histological results in an implant retrieved 4 years after insertion. A case report. *J. Periodontol.* **2004**, *75*, 1161–1166. [CrossRef]
26. Stavropoulos, A.; Karring, T. Guided tissue regeneration combined with a deproteinized bovine bone mineral (Bio-Oss®) in the treatment of intrabony periodontal defects: 6-year results from a randomized-controlled clinical trial. *J. Clin. Periodontol.* **2010**, *37*, 200–210. [CrossRef] [PubMed]
27. Lelovas, P.P.; Xanthos, T.T.; Thoma, S.E.; Lyritis, G.P.; Dontas, I.A. The laboratory rat as an animal model for osteoporosis research. *Comp. Med.* **2008**, *58*, 424–430.
28. Durão, S.; Gomes, P.S.; Colaço, B.; Silva, J.; Fonseca, H.; Duarte, J.; Felino, A.; Fernandes, M.H. The biomaterial-mediated healing of critical size bone defects in the ovariectomized rat. *Osteoporos. Int.* **2014**, *25*, 1535–1545. [CrossRef] [PubMed]
29. Ahmed, A.G.; Awartani, F.A.; Niazy, A.A.; Jansen, J.A.; Alghamdi, H.S. A Combination of Biphasic Calcium Phosphate (Maxresorb®) and Hyaluronic Acid Gel (Hyadent®) for Repairing Osseous Defects in a Rat Model. *Appl. Sci.* **2020**, *10*, 1651. [CrossRef]
30. Van Houdt, C.I.; Tim, C.R.; Crovace, M.C.; Zanotto, E.D.; Peitl, O.; Ulrich, D.J.; Jansen, J.A.; Parizotto, N.A.; Renno, A.C.; van den Beucken, J.J. Bone regeneration and gene expression in bone defects under healthy and osteoporotic bone conditions using two commercially available bone graft substitutes. *Biomed. Mater.* **2015**, *10*, 035003. [CrossRef] [PubMed]
31. Jensen, S.S.; Aaboe, M.; Pinholt, E.M.; Hjorting-Hansen, E.; Melsen, F.; Ruyter, I.E. Tissue reaction and material characteristics of four bone substitutes. *Int. J. Oral Maxillofac Implant.* **1996**, *11*, 55–66.
32. Berglundh, T.; Lindhe, J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. *Clin. Oral Implant. Res.* **1997**, *8*, 117–124. [CrossRef] [PubMed]
33. Pinholt, E.M.; Bang, G.; Haanaes, H.R. Alveolar ridge augmentation in rats by Bio-Oss. *Eur. J. Oral Sci.* **1991**, *99*, 154–161. [CrossRef] [PubMed]
34. Tapety, F.I.; Amizuka, N.; Uoshima, K.; Nomura, S.; Maeda, T. A histological evaluation of the involvement of Bio-Oss® in osteoblastic differentiation and matrix synthesis. *Clin. Oral Implant. Res.* **2004**, *15*, 315–324. [CrossRef] [PubMed]
35. Turner, A.S. Animal models of osteoporosis-necessity and limitations. *Eur Cell Mater.* **2001**, *1*, 13.
36. Sanfilippo, F.; Bianchi, A.E. Osteoporosis: The effect on maxillary bone resorption and therapeutic possibilities by means of implant prostheses-a literature review and clinical considerations. *Int. J. Periodontics Restor. Dent.* **2003**, *23*, 447–457. [CrossRef]
37. Blomqvist, J.E.; Alberius, P.; Isaksson, S.; Linde, A.; Hansson, B.-G.R. Factors in implant integration failure after bone grafting: An osteometric and endocrinologic matched analysis. *Int. J. Oral Maxillofac. Surg.* **1996**, *25*, 63–68. [CrossRef]
38. Khosla, S.; Oursler, M.J.; Monroe, D.G. Estrogen and the skeleton. *Trends Endocrinol. Metab.* **2012**, *23*, 576–581. [CrossRef] [PubMed]
39. Väänänen, H.K.; Härkönen, P.L. Estrogen and bone metabolism. *Maturitas* **1996**, *23*, S65–S69. [CrossRef]
40. Teófilo, J.M.; Brentegani, L.G.; Lamano-Carvalho, T.L. Bone healing in osteoporotic female rats following intra-alveolar grafting of bioactive glass. *Arch. Oral Biol.* **2004**, *49*, 755–762. [CrossRef]
41. Luize, D.S.; Bosco, A.F.; Bonfante, S.; de Almeida, J.M. Influence of ovariectomy on healing of autogenous bone block grafts in the mandible: A histomorphometric study in an aged rat model. *Int. J. Oral. Maxillofac. Implant.* **2008**, *23*, 207–214.
42. Hayashi, K.; Uenoyama, K.; Matsuguchi, N.; Nakagawa, S.; Sugioka, Y. The affinity of bone to hydroxyapatite and alumina in experimentally induced osteoporosis. *J. Arthroplast.* **1989**, *4*, 257–262. [CrossRef]
43. Hayashi, K.; Uenoyama, K.; Mashima, T.; Sugioka, Y. Remodelling of bone around hydroxyapatite and titanium in experimental osteoporosis. *Biomaterials* **1994**, *15*, 11–16. [CrossRef]
44. Fuegl, A.; Tangl, S.; Keibl, C.; Watzek, G.; Redl, H.; Gruber, R. The impact of ovariectomy and hyperglycemia on graft consolidation in rat calvaria. *Clin. Oral. Implant. Res.* **2011**, *22*, 524–529. [CrossRef] [PubMed]
45. He, Y.-X.; Zhang, G.; Pan, X.-H.; Liu, Z.; Zheng, L.-Z.; Chan, C.-W.; Lee, K.-M.; Cao, Y.-P.; Li, G.; Wei, L. Impaired bone healing pattern in mice with ovariectomy-induced osteoporosis: A drill-hole defect model. *Bone* **2011**, *48*, 1388–1400. [CrossRef]
46. Rolvien, T.; Barbeck, M.; Wenisch, S.; Amling, M.; Krause, M. Cellular mechanisms responsible for success and failure of bone substitute materials. *Int. J. Mol. Sci.* **2018**, *19*, 2893. [CrossRef] [PubMed]