Received: 23 July 2020 | Revised: 29 September 2020 | Accepted: 27 October 2020

DOI: 10.1002/JPER.20-0547

JOURNAL OF Periodontology

ORIGINAL ARTICLE

Microstructural volumetric analysis of the jaw following dental implantation under systemic bisphosphonate delivery: An in vivo and ex vivo rat study

Kristian Kniha[1,2] | **Anna Bock**[2] | **Florian Peters**[2] | **Zuzanna Anna Magnuska**[3] | **Felix Gremse**[3] | **Stephan Christian Möhlhenrich**[4] | **Frank Hölzle**[2] | **Ali Modabber**[2]

[1] Private clinic for oral and maxillofacial surgery, KnihaSchlegel and colleagues, Munich, Germany

[2] Department of Oral and Cranio-Maxillofacial Surgery, University Hospital RWTH Aachen, Aachen, Germany

[3] Department of Experimental Molecular Imaging, RWTH Aachen University, Aachen, Germany

[4] Department of Orthodontics, University of Witten/Herdecke, Witten, Germany

Correspondence
Priv. Doz. Dr. med. dent. Kristian Kniha, Private clinic for oral and maxillofacial surgery, Kniha, Schlegel and colleagues, Arnulfstraße 19, 80335 Munich, Germany.
Email:info@kniha-schlegel.de

Abstract

Background: Because of bisphosphonate medication, dental implantation with a subsequent infection poses a relevant risk factor to suffer from medication-related osteonecrosis of the jaw. This rat study evaluated different implant materials under systemic bisphosphonate delivery using micro-computed tomography (μCT) images.

Methods: Fifty-four rats were randomly allocated into a control group 1, test group 2 with intravenous drug application of zoledronic acid and test group 3 with a subcutaneous application of alendronic acid. After 4 weeks of drug delivery, the first molar on each side of the upper jaw was extracted, and either a zirconia or a titanium implant was immediately inserted. Radiological examinations at four timepoints before the operation, 1 week later, 6 weeks later and after 12 weeks of follow up included μCT measurements of the in vivo peri-implant bone loss. μCT measurements of the ex vivo peri-implant bony structure after 12 weeks follow-up covered the bone mineral density, -volume, -trabecular thickness and -separation.

Results: Both test groups showed a significant increase in bone loss over time ($P < 0.05$). The clinical observations of exposed bone revealed that most cases occurred under alendronic acid delivery. Exposed bone was recorded only in the test groups around both titanium and zirconia implants. Regarding the peri-implant bony structure, no significant differences were found between both materials.

Conclusions: Systemic bisphosphonate delivery led to increased peri-implant bone loss over time after immediate implant insertion. In terms of bone resorption and bone quality parameters, no implant material was superior to the other.

KEYWORDS
dental implants, necrosis, titanium, X-ray microtomography, zirconium oxide

1 | INTRODUCTION

Bisphosphonates are a class of drugs that prevent the loss of bone density, and they are mainly used to treat osteoporosis and multi-morbid patients with osseous metastases of solid tumors.[1,2] Aside from having a therapeutic effect of reducing the spread of bone metastases or stopping bone osteolysis, the use of antiresorptive drugs often

JOURNAL OF Periodontology

leads to side effects in the form of osteonecrosis of the jaw.[3,4]

Osteonecrosis is not a specific disease entity but is the final common pathway of several conditions that lead to bone death.[5] Antiresorptive jaw necrosis is defined as an exposed jaw bone for a few weeks with a corresponding anamnesis of antiresorptive drugs.[6,7]

In most antiresorptive-associated jaw necrosis, the triggers in the oral cavity are identifiable. These triggers include periodontally diseased teeth, denture pressure points, and dental surgery interventions, such as tooth extractions, with germ migration into the jaw bone.[8] Another source of infection for jaw necrosis is the sulcular soft tissue around a dental implant.[9] On one hand, implants can theoretically trigger an inflammatory reaction during insertion. On the other hand, implants can contribute to avoiding denture pressure points by reducing the load on the gingiva. Subsequently, this may also reduce the individual risk of osteonecrosis.

Titanium implants are the gold standard with a high amount of long-term data, whereas zirconia has recently become an alternative implant material.[10–13] Long-term studies on novel zirconium dioxide implants are scarce, although the initial clinical results were promising.[14]

Materials with low affinity values for bacteria could minimize the risk of implant bed inflammation, jaw necrosis, or faulty osseointegration in patients with antiresorptive medications. Clever et al. showed that the soft tissue around titanium implants clinically developed a stronger inflammatory response to experimental plaque accumulation compared with that around zirconia implants.[15,16]

This in vivo rat primarily aimed to evaluate changes between two different implant materials regarding the extent of bone loss and jaw necrosis with micro-computed tomography (μCT) images following dental implantation under a systemic bisphosphonate delivery. In this study design, zirconia implants were compared with titanium implants, which were immediately inserted after tooth extraction. The second aim was to assess bone changes around implants under two types of bisphosphonates.

2 | MATERIALS AND METHODS

2.1 | Experimental protocol

Fifty-four adult male rats[*] with a weight of 250 g and an age of 7 weeks at the beginning of the experiment were used in this study. The study protocol was approved by the appropriate local authority[†]. This manuscript contains the investigations that refer to the radiological results of this study ant it was conducted in accordance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments)[17] and the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

The rats were randomly allocated into three groups. One control group and two experimental groups were divided as follows: control group (group 1, no drug application, each $n = 18$), zoledronic acid (test group 2, intravenous drug application, N = 18), and alendronic acid (test group 3, subcutaneous drug application, $n = 18$). Systemic medication with antiresorptive drugs was conducted for a period of 4 months and was started 4 weeks before implantation (Figure 1A). Group 2 received a dose of 0.04 mg/kg body weight zoledronic acid[‡] intravenously in the tail vein once every week.[18] A total of 0.2 mg/kg body weight alendronic acid[§] was applied subcutaneously five times a week in group 3.[19] Before application, the bisphosphonates were diluted with physiologic phosphate-buffered saline. The control groups received no medication. The rats were provided with food and water ad libitum, with only soft soaked food administered after implantation until the end of the investigation.

After every μCT scan, the peri-implant mucosa in all rats was clinically inspected after each postoperative scan for medication-related osteonecrosis of the jaw (MRONJ) lesions, such as exposed jaw bone (Figure 1C).

2.2 | Implant surgery

Microrough titanium and zirconia implants with a polished shoulder ($n = 54$ each material, length of 4 mm and diameter of 2 mm) were custom-made by a company[**] with the same process used on commercially available implants.

After 4 weeks of drug delivery, the rats received an intraperitoneal anesthetic cocktail consisting of 90 mg/kg body weight ketamine[††] and 0.2 mg/kg body weight medetomidine hydrochloride[‡‡]. The first molar on each side of the upper jaw was gently extracted with forceps. The mesial root sockets were then inspected, and the remaining tooth fragments were removed. Subsequently, either a zirconia or a titanium immediate implant was used ran-

[*] (Sprague-Dawley rats, Janvier Labs, Le Genest-Saint-Isle, France).

[†] (IRB approval: Landesamt für Natur und Verbraucherschutz, Recklinghausen, Germany; Ref. 2018A314).

[‡] (Zoledronic acid, Mylan dura GmbH, Darmstadt, Germany).

[§] (Alendronate sodium trihydrate, Sigma Aldrich GmbH, Munich, Germany).

[**] (Straumann GmbH, Basel, Switzerland).

[††] (Ketamine, Medistar GmbH, Ascheberg, Germany).

[‡‡] (Domitor, Bayer Austria, Wien, Austria).

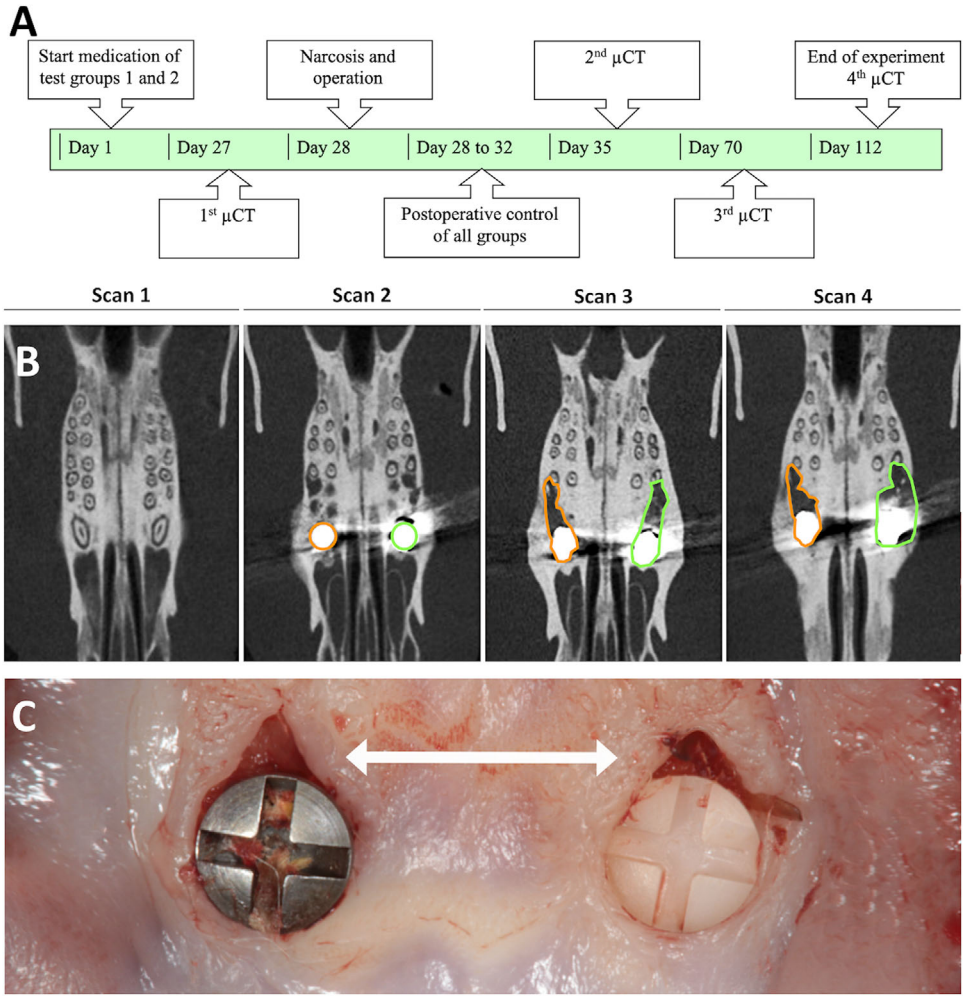

FIGURE 1 (**A**) This image shows the timeline of this study (**B**) One case with increasing peri-implant bone loss after surgery (scans 2–4) is presented. The volumetric bone loss measurements were carried out around both implants (titanium implant on the right side and zirconia implant on the left side). (**C**) After immediate implant placement, the animals were clinically inspected after each scan. In this case of the zoledronic acid group, exposed bone was visible next to both implants (arrow: titanium implant on the left side and zirconia implant on the right side)

domly on one side each. A split-mouth design was used with respect to the implant material and the zirconia- or titanium material was randomly distributed on both maxillary sides. The insertion process included a pilot drill with a 2.2 mm diameter **, and a marker on the drill ensured that each implant site was prepared at the same depth with a strict saline irrigation. The implants were inserted with a screwdriver and a torque of 15 to 20 Ncm, with the shoulder at the level of the mucosa using a transgingival healing process. All implants were controlled for primary stability and for any occlusal interfering contacts. No sutures were needed in this flapless approach. At the end of the surgery, the antidote atipamezole hydrochloride[§§], with a dose of 0.8 mg/kg body weight, was applied subcutaneously to keep the duration of the operation as short as possible. In the first 3 days postoperatively, the animals were visited by the investigator or his representative once a day (several times a day if necessary) and treated once a day with Carprofen 4 mg/kg subcutaneously[***] according to a score sheet.

2.3 | Radiological examination

2.3.1 | In vivo volumetric μCT measurements of peri-implant bone loss

A total of 208 in vivo μCT scans were conducted and evaluated by Z.M. investigator. As the baseline (first scan) 1 day before implant surgery, the first radiological

[§§] (Atipamezole hydrochloride, Orion Pharma, Espoo, Finland).

[***] (Rimadyl, Zoetis GmbH, Berlin, Germany)

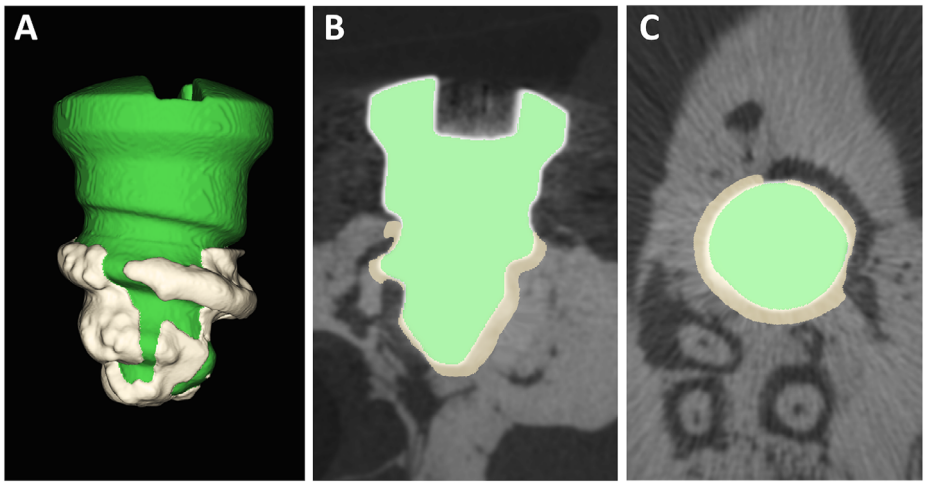

FIGURE 2 (**A**) (**B**) and (**C**): Standardized ex vivo skeletal morphometric microstructure measurements of the peri-implant bone at the end of the investigation

examination in the in vivo μCT[†††] was carried out under inhalation anesthesia with Isofluran[‡‡‡]. After positioning the animal in the rat bed, the head of a rat was scanned with an ultra-focus magnification through 360° of rotation at a 0.75° increment with 0.3 s/degree. The μCT data were reconstructed at an isotropic voxel size of 40 μm. For analysis, the μCT data was down-sampled using binning to a voxel size 80 μm. The voxel resolution (size) used for ex vivo scans was 15 μm x 15 μm x 15 μm.

After implant surgery, three more in vivo μCT scans were performed. After the operation, the second scan was performed 1 week later, the third scan 6 weeks later and the fourth scan 12 weeks later. Image analysis was performed with the μCT evaluation software[§§§].[20] The volumetric measurements of bone loss were conducted after implantation (second to fourth μCT) using segmented μCT data (Figure 1B). Including the implant body, 3D bone loss was measured in mm^3 for each implant side in each animal using the axial, coronal, and sagittal planes. To evaluate the reproducibility of the bone loss measurement, a randomly selected animal was used, and the bone loss measurement in both implant types was repeated 10 times on different days at different times by one investigator.

2.3.2 | Ex vivo skeletal morphometric microstructure measurements of the peri-implant bony structure

At the end of the investigation, 59 ex vivo μCT scans[†††] were carried out. Owing to implant loss, 29 titanium and 30 zirconia implants could be enrolled for this measurement (Figure 2). Image analysis was performed using a software[****]. In the first step, an implant was segmented in the image by thresholding. Afterwards, a coat of fixed 10 mm thickness was computed around the segment using morphological operations. Then, the bone tissue was segmented within the coat volume.[21] The segmentation of hard tissue using the standardized process enabled the evaluation of bone mineral density (BMD in g/cm3), bone volume per total volume (BV/TV in %), trabecular thickness (Tb.Th in mm), and trabecular separation (Tb.Sp in cm).

2.4 | Statistical analysis

Analyses were performed using a software[††††]. The analysis values were tested for normal distribution using the Shapiro-Wilk normality test. The groups were analyzed using the multiple *t* test, which is a two-stage linear setup introduced by Benjamini, Krieger, and Vekutieli,[22] with Q = 1% and each row being analyzed individually without assuming a consistent standard deviation. The in vivo μCT bone loss measurements were analyzed using a two-way ANOVA with a Geisser-Greenhouse correction. Post hoc Tukey's multiple comparison test, with individual variances computed for each comparison, was also conducted. The ex vivo evaluation used an unpaired multiple *t* test. We assessed any effect in the statistical model as significant if the corresponding *P*-value fell below the 5% margin.

[†††] (U-CT OI, MILabs, Utrecht, The Netherlands).
[‡‡‡] (Isofluran, 2.5–5 vol.% Piramal GmbH, Hallbergmoos, Germany).
[§§§] (Imalytics Preclinical, Gremse-IT GmbH, Aachen, Germany)

[****] (Skyscan 1272, Bruker μCT, Billerica, MA, USA)
[††††] (Prism 8, GraphPad, La Jolla, CA, USA)

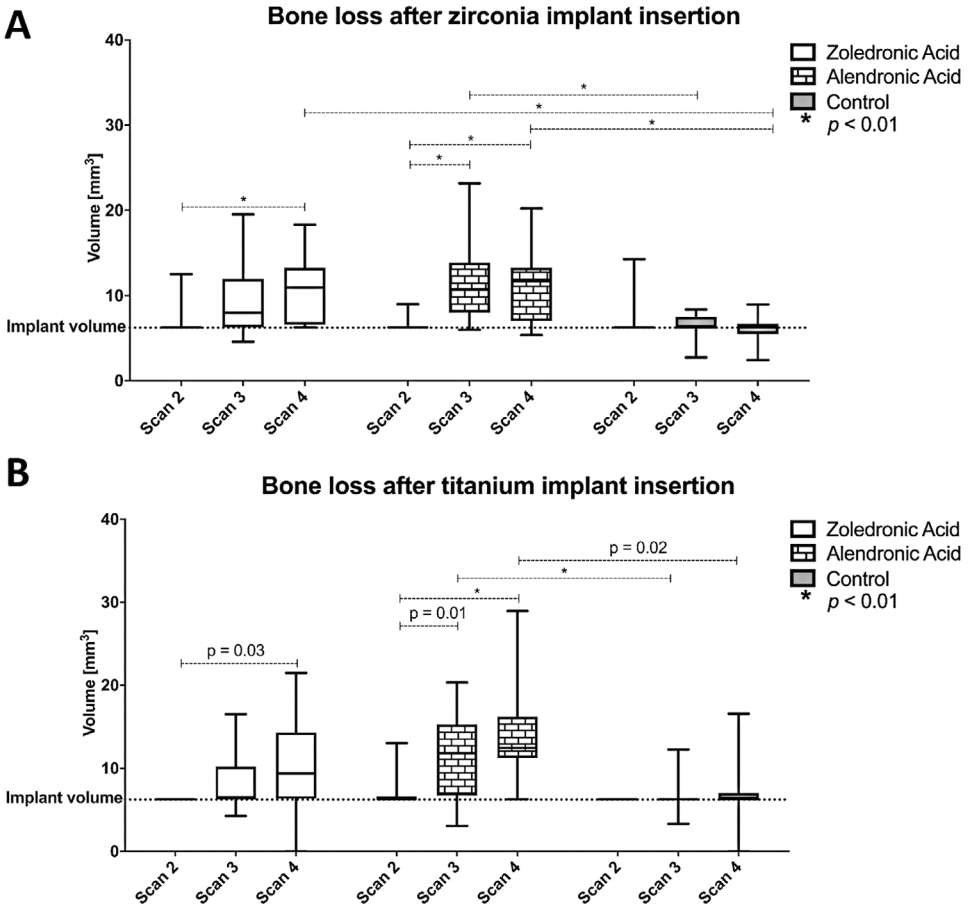

FIGURE 3 In vivo volumetric μCT measurements of peri-implant bone loss around **A** zirconia and **B** titanium

Post-hoc power analysis was performed with a software[‡‡‡‡] using the F-test ANOVA to determine the power of 99% (primary study aim) based on the total sample size of 70 measurements and a number of six groups (groups 1 to 3 for each titanium and zirconia implants) at scan four using an effect size of 0.58 and an α of 0.05 (Mean 1: 10.02, mean 2: 10.46, mean 3: 14.31, mean 4: 11.33, mean 5: 7.35, mean 6: 6.19).

3 | RESULTS

Of 54 animals, a total of 52 rats could be included to the radiological evaluation. Two animals of group 2 were unfortunately lost, one in the course of the operation during anesthesia probably to respiratory arrest and the second animal during medication in the rat restrainer. Group 1 presented 51,5% of implant loss, group 2 15,7% and group 3 44,4%.

[‡‡‡‡] (G Power software, Heinrich-Heine-Universität, Düsseldorf, Germany)

3.1 | In vivo volumetric μCT measurements of peri-implant bone loss

Both test groups (zoledronic acid and alendronic acid application) around both implant materials showed a significant increase in bone loss over time ($P < 0.05$, Figures 3A and 3B). By contrast, control group showed relatively low bone resorption values. In cases of implant loss, a decrease of bone loss below the defined implant volume was measured, which could be evaluated especially in the control groups. The comparison between titanium and zirconia implants revealed no significant differences over time in terms of bone resorption (Table 1).

The clinical observations of exposed bone revealed that most cases occurred in test group 3 (alendronic acid, $n = 24$), followed by test group 2 (zoledronic acid, $n = 15$). Exposed bone was recorded only in the test groups around both titanium and zirconia implants, with the control group showing no exposed bone at all (Table 1).

To evaluate the reproducibility of the bone loss measurement, a randomly selected animal was used, and the bone loss measurement in both implant types was repeated 10 times on different days at different times by

TABLE 1 **A**: In vivo investigation of 3D radiological peri-implant bone loss and clinical osteonecrosis observation. **B**: Descriptive data of the ex vivo skeletal morphometric microstructure measurements of the peri-implant bony structure and in vivo bone quality changes over time. *SD = standard deviation, MIN = minimum, MAX = maximum, CV = coefficient of variation, N = number of measurements, Zol = zoledronic acid, Ale = alendronic acid, C1 = control group 1 and C2 = control group 2

A	**Mean (SD)**								
Group	**Zoledronic acid**			**Alendronic acid**			**Control**		
Number of rats	**16**			**18**			**18**		
	Ti	Zi	*p**	Ti	Zi	*p**	Ti	Zi	*p**
Clinical observation: osteonecrosis	8	7		12	12		0	0	
Bone loss scan 2 (mm^3)	6.28 (0.00)	7.15 (2.00)	ns	7.19 (1.98)	6.50 (0.68)	ns	6.28 (0.00)	7.04 (2.26)	ns
Bone loss scan 3 (mm^3)	8.10 (3.36)	9.55 (4.35)	ns	11.22 (5.02)	11.60 (4.64)	ns	6.53 (1.67)	6.42 (1.42)	ns
Bone loss scan 4 (mm^3)	10.02 (5.43)	10.46 (3.59)	ns	14.31 (9.67)	11.33 (4.26)	ns	7.35 (3.63)	6.19 (1.61)	ns

B	**Bone mineral density**			**Bone volume fraction**			**Trabecular thickness**			**Trabecular separation**		
Ex vivo measurements of the peri-implant bone												
	Titanium											
	Zol	Ale	Con	Zol	Ale	Con	Zol	Ale	Con	Zol	Ale	Con
N	15	10	4	15	10	4	15	10	4	15	10	4
Mean	2.93	2.44	1.87	56.0	34.1	24.7	0.07	0.08	0.07	0.08	0.12	0.11
SD	1.04	0.60	1.50	38.4	31.1	25.7	0.03	0.01	0.01	0.06	0.05	0.08
Ex vivo measurements of the peri-implant bone												
	Zirconia											
	Zol	Ale	Con	Zol	Ale	Con	Zol	Ale	Con	Zol	Ale	Con
N	11	9	10	11	9	10	11	9	10	11	9	10
Mean	3.18	2.29	2.98	69.0	28.8	67.3	0.08	0.08	0.05	0.05	0.12	0.06
SD	0.82	0.78	1.41	32.8	27.8	36.8	0.03	0.03	0.02	0.05	0.04	0.07

*P values refer to the comparison between titanium and zirconia implants within each group.

†Ale, Alendronic Acid; Con, control group; CV, coefficient of variation; MAX, maximum; MIN, minimum; N, number of measurements; SD, standard deviation; Zol, Zoledronic Acid.

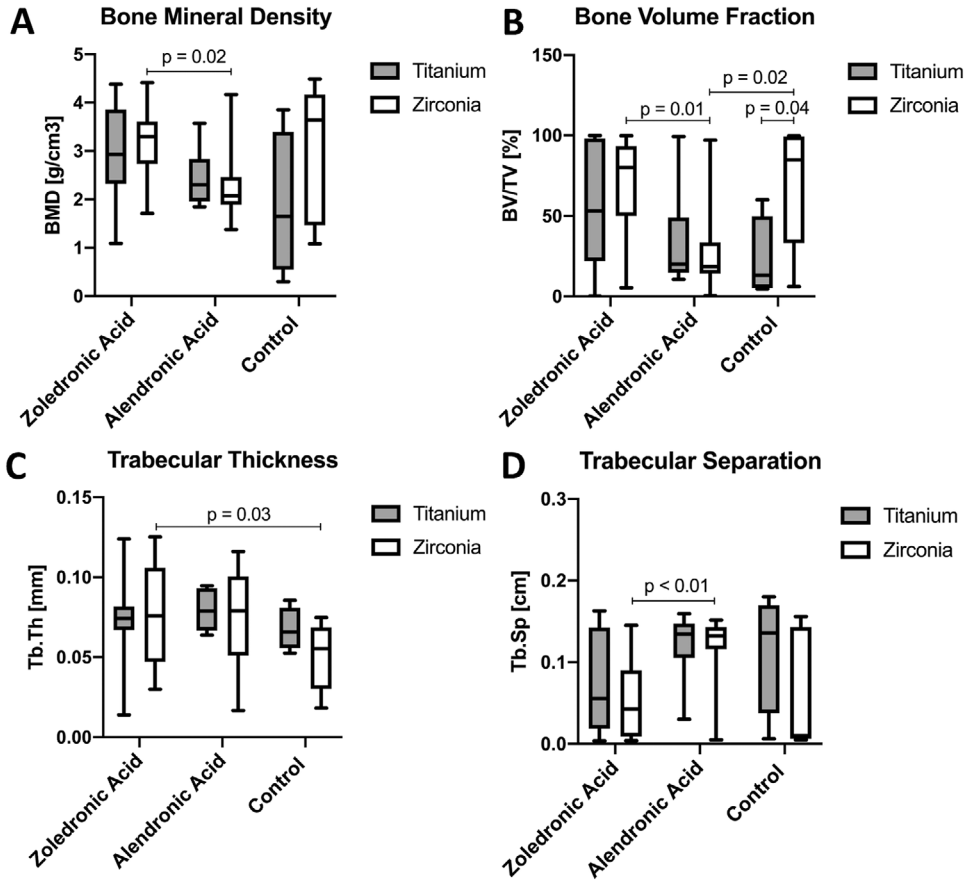

FIGURE 4 Ex vivo skeletal morphometric microstructure measurements of the peri-implant bony structure. Figure shows (**A**) the bone mineral density, (**B**) the bone volume fraction, (**C**) the trabecular thickness, and (**D**) the trabecular separation

one investigator. The reproducibility of the bone loss segmentation showed a coefficient of variation of 0.09 (mean: 16.98) around the titanium implants and 0.07 (mean: 16.25) around the zirconia implants (see Table S1 in online Journal of Periodontology).

3.2 | Ex vivo skeletal morphometric microstructure measurements of the peri-implant bony structure

In the zirconia implant group, the measured BMD values were lower in the alendronic acid group than in test group 2 (zoledronic acid, $P = 0.02$, Figure 4A). By contrast, no significant difference in the BMD was measured in the titanium implant group and between the implant materials.

Between implant materials in the control group, zirconia presented significantly higher BV/TV values ($P = 0.04$, Figure 4B). In terms of bone volume around the material zirconia, the alendronic acid delivery significantly showed lower values compared with the rest of the groups ($P = 0.01$ and $P = 0.02$).

The within-group comparison of the Tb.Th values of the zirconia implants achieved statistical significance between the zoledronic acid test group and control group ($P = 0.03$), but these discrepancies failed in significance in the other groups (Figure 4C). The Tb.Th values of the titanium implants appeared to be balanced in all groups, as the comparison failed to achieve statistical significance ($P > 0.05$).

In the within-group comparison with higher Tb.Sp values, the zirconia implants reached statistical significance in the alendronic acid group compared with test group 2 ($P < 0.01$), whereas a lack of significance was observed around the titanium implants. Overall, the Tb.Sp values seemed to be slightly higher around the titanium material (Figure 4D).

4 | DISCUSSION

The study results showed that systemic bisphosphonate delivery led to increased peri-implant bone loss over time after immediate implant insertion. In terms of bone resorption and bone quality parameters, no implant material was superior to the other.

JOURNAL OF Periodontology

Owing to the isotropic voxel sizes and calibrated voxel units, volumetric μCT data are suitable for quantitative analysis, such as the investigation of jaw necrosis.[20,23] μCT data can be used for high-resolution 3D imaging of in vivo and ex vivo laboratory settings.[24,25] Bone morphology and calcifications provide a particularly strong native contrast that allows the analysis of the microarchitecture of the bone and the quantification of hard tissue.[26,27] Ionizing radiation dose of μCT could have had an influence on the study outcome, that is, animal survival.[28] μCT scanning doses are often higher when compared to clinical CT scans because of the smaller volumes and lower signal per voxel. In the present study, each animal was scanned four times in a period of 3.5 months, in contrast to other longitudinal in vivo studies where animals can receive up to 11 scans over 2 days without apparent symptoms.[29] Moreover, the performed μCT scans were restricted only to the head and skull area which is relatively insensitive to irradiation. Each rat received a cumulative radiation dose of approximately two Grey over the course of investigation. Zhai et al. showed that the damage of bone tissue in rats, caused by a single dose of two Grey irradiation, is reversible and it is likely to recover completely.[30] Based on the score sheets we were able to control the general well-being of the animals. No radiation related effects were noticed.

The study design aimed to simulate and analyze a high-risk group for dental implantation because of a systemic bisphosphonate medication in the rat model using a high application rate. We applied a systemic zoledronic acid and alendronic acid dose per body weight comparable with that of humans. We recognize that pathophysiology can vary between our rat model and humans. Osteonecrosis develops in areas where an infection or inflammation was present (e.g. periodontitis, endodontic lesion). However, in this manuscript, the tooth was extracted in a healthy condition. In humans, it is necessary to determine whether the risk of suffering osteonecrosis through implantation is greater than the benefit of avoiding pressure points on the prosthesis.[31] Intravenous administration, as applied in this study, is associated with a higher risk than oral administration (i.v. – > oral application).[8] Furthermore, the frequency and duration of the medication increase the incidence of osteonecrosis.[8] Based on this information, we decided to start the weekly drug application 4 weeks before surgery.

In this study, the effects of zirconium and titanium as implant materials on the risk of osteonecrosis without the influence of antibiotic shielding were investigated. Only the effect of the implant material on the incidence of bone loss and exposed bone was evaluated and not the antibiotic effect. The comparison between titanium and zirconia implants revealed no significant differences over time in terms of bone resorption. With regard to the benefit of perioperative antibiotic prophylaxis in humans, the data situation is relatively clear, and thus antibiotic screening should be performed in these cases.[8]

Du et al. showed that the first molar of the rat maxilla can be used as a site for research into the osseointegration of dental implants.[32] They concluded that at least 4 weeks of healing should be considered for evaluating the degree of osseointegration.

To not severely compromise the blood supply of bone in cases with antiresorptive drugs, it is recommended to operate atraumatically and to denude the periosteum as little as possible. Currently, there is no clear data on whether closed or open implant healing is advantageous in humans, as a closed procedure requires further surgical intervention during implant exposure.[31] Based on this information, we decided to proceed to an open healing procedure. In our study, a comparatively increasing peri-implant bone loss rate was observed in the test groups and control group showed relatively low bone resorption values. Especially under alendronic acid medication by contrast, the animals showed steadily increasing resorption rates.

Furthermore, patients with antiresorptive medication should perform the best possible oral hygiene after implantation. However, because of the preclinical study design of this rat study, no specific oral hygiene could be performed after surgery. Additionally, compared with delayed implantation, a riskier immediate implantation (i.e. simultaneous tooth extraction and implantation) was carried out. Therefore, increased inflammation rates and implant loss compared with a conservative implant procedure could be assumed. This might explain why a total of 29 titanium and 30 zirconia implants were lost at the end of the experiment. A further explanation for the increased implant loss rate in the healthy control group could possibly be a better and faster reacting bone metabolism and thus more pronounced inflammatory reaction to a high-risk implant procedure. Furthermore, after implant loss the sound bone showed a superior remodeling and bone growth behavior.

The clinical observations of this study showed that exposed bone occurred only in test groups 2 and 3. In these cases, exposed bone was recorded equally around both titanium implants and zirconia implants, with the control group showing no exposed bone at all.

In rats in another study, alendronic acid decreased bone formation and vascularity within the root socket.[33] Aguirre et al. found that such effects could also contribute to ONJ lesion development.[33] Abtahi et al. showed that bisphosphonate-related jaw osteonecrosis tended to develop first after exposure of the bone when large ONJ-like lesions were evaluated after dental removal[34] and that the group receiving a high dose of systemic bisphosphonate (alendronate) was the group showing the most bone loss on μCT.[34]

Zoledronate is the most potent and nitrogen-containing bisphosphonate, and it is more active than alendronate.[35,36] The groups under alendronate medication showed higher BMD values than the other groups in ovariectomized rats.[37,38] Compared with the group without zoledronate application, the group with this medication according to Choi et al. showed significantly increased BV/TV values.[39] These results agree with our study, as zoledronic acid presented increased in vivo and ex vivo BMD and BV/TV values compared with alendronic acid. A critical reflection on this study revealed that the split mouth design was mainly lost as several animals lost one implant. Furthermore, the small amount of scattering could have interfered in the image analysis when comparing titanium and zirconia implants.

5 | CONCLUSIONS

A systemic bisphosphonate delivery led to increased bone loss over time after immediate implant insertion. Exposed bone and MRONJ lesions were recorded only in the test groups equally around both titanium implants and zirconia implants, with the control group showing no exposed bone at all. In terms of bone resorption and bone quality parameters, no implant material was superior to the other.

ACKNOWLEDGMENTS

The authors thank Straumann (Straumann GmbH, Basel, Switzerland) for the fabrication of dental mini-implants.

CONFLICT OF INTEREST

Kristian Kniha received a research grant and a speaker honorarium from the Straumann Company, but neither was related to this study. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

KK: conception and design, acquisition of data, drafting the work, final approval; AB: conception and design, drafting the work, final approval; FP: analysis and interpretation, revising the work, final approval; ZM: acquisition of data, revising the work, final approval; FG: conception and design, revising the work, final approval; SM: analysis and interpretation, revising the work, final approval; FH: analysis and interpretation, revising the work, final approval; AM: conception and design, analysis and interpretation, drafting the work, final approval; Each author participated sufficiently in the work to take public responsibility for appropriate portions of the content; Each author agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Eckert AW, Maurer P, Meyer L, et al. Bisphosphonate-related jaw necrosis–severe complication in maxillofacial surgery. *Cancer Treat Rev*. 2007;33:58-63.
2. Russell RG. Bisphosphonates: the first 40 years. *Bone*. 2011;49:2-19.
3. Matsuo A, Hamada H, Takahashi H, Okamoto A, Kaise H, Chikazu D. Evaluation of dental implants as a risk factor for the development of bisphosphonate-related osteonecrosis of the jaw in breast cancer patients. *Odontology*. 2016;104:363-371.
4. Fliefel R, Troltzsch M, Kuhnisch J, Ehrenfeld M, Otto S. Treatment strategies and outcomes of bisphosphonate-related osteonecrosis of the jaw (BRONJ) with characterization of patients: a systematic review. *Int J Oral Maxillofac Surg*. 2015;44:568-585.
5. Fondi C, Franchi A. Definition of bone necrosis by the pathologist. *Clin Cases Miner Bone Metab*. 2007;4:21-26.
6. Vogl T, Kellermann O, Randzio J, et al. Results of magnetic resonance tomography of the temporomandibular joint using optimized surface coils. *Rofo* article in German. 1988;149:502-507.
7. Akashi M, Kusumoto J, Takeda D, Shigeta T, Hasegawa T, Komori T. A literature review of perioperative antibiotic administration in surgery for medication-related osteonecrosis of the jaw. *Oral Maxillofac Surg*. 2018;22:369-378.
8. Walter C, Al-Nawas B, Wolff T, Schiegnitz E, Grötz KA. Dental implants in patients treated with antiresorptive medication – a systematic literature review. *Int J Implant Dent*. 2016;2:9.
9. Holzinger D, Seemann R, Matoni N, Ewers R, Millesi W, Wutzl A. Effect of dental implants on bisphosphonate-related osteonecrosis of the jaws. *J Oral Maxillofac Surg*. 2014;72:1937.e1931-1938.
10. Sivaraman K, Chopra A, Narayan AI, Balakrishnan D. Is zirconia a viable alternative to titanium for oral implant? A critical review. *J Prosthodont Res*. 2018;62:121-133.
11. Cionca N, Hashim D, Mombelli A. Zirconia dental implants: where are we now, and where are we heading?. 2017:241-258.
12. Kniha K, Schlegel KA, Kniha H, Modabber A, Holzle F, Kniha K. Evaluation of peri-implant bone levels and soft tissue dimensions around zirconia implants-a three-year follow-up study. *Int J Oral Maxillofac Surg*. 2018;47:492-498.
13. Kniha K, Modabber A, Kniha H, Mohlhenrich SC, Holzle F, Milz S. Dimensions of hard and soft tissue around adjacent, compared with single-tooth, zirconia implants. *Br J Oral Maxillofac Surg*. 2018;56:43-47.
14. Kniha K, Schlegel KA, Kniha H, Modabber A, Neukam F, Kniha K. Papilla-crown height dimensions around zirconium dioxide implants in the esthetic area: a 3-year follow-up study. *J Prosthodont*. 2019;28:e694-e698.
15. Clever K, Schlegel KA, Kniha H, et al. Experimental peri-implant mucositis around titanium and zirconia implants in comparison to a natural tooth: part 1-host-derived immunological parameters. *Int J Oral Maxillofac Surg*. 2019;48:554-559.
16. Clever K, Schlegel KA, Kniha H, et al. Experimental peri-implant mucositis around titanium and zirconia implants in comparison to a natural tooth: part 2-clinical and microbiological parameters. *Int J Oral Maxillofac Surg*. 2019;48:560-565.
17. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLOS Biology*. 2020;18:e3000410.

18. Abtahi J, Agholme F, Sandberg O, Aspenberg P. Effect of local vs. systemic bisphosphonate delivery on dental implant fixation in a model of osteonecrosis of the jaw. *J Dent Res*. 2013;92:279-283.
19. Bernhardsson M, Sandberg O, Aspenberg P. Anti-RANKL treatment improves screw fixation in cancellous bone in rats. *Injury*. 2015;46:990-995.
20. Gremse F, Stark M, Ehling J, Menzel JR, Lammers T, Kiessling F. Imalytics preclinical: interactive analysis of biomedical volume data. *Theranostics*. 2016;6:328-341.
21. Becker K, Jandik K, Stauber M, Mihatovic I, Drescher D, Schwarz F. Microstructural volumetric analysis of lateral ridge augmentation using differently conditioned tooth roots. *Clin Oral Investig*. 2019;23:3063-3071.
22. Benjamini Y, Krieger AM, Yekutieli D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika*. 2006;93:491-507.
23. Kamal M, Gremse F, Rosenhain S, et al. Comparison of bone grafts from various donor sites in human bone specimens. *J Craniofac Surg*. 2018;29:1661-1665.
24. Ehling J, Babickova J, Gremse F, et al. Quantitative micro-computed tomography imaging of vascular dysfunction in progressive kidney diseases. *J Am Soc Nephrol*. 2016;27:520-532.
25. Gremse F, Theek B, Kunjachan S, et al. Absorption reconstruction improves biodistribution assessment of fluorescent nanoprobes using hybrid fluorescence-mediated tomography. *Theranostics*. 2014;4:960-971.
26. Ehling J, Bartneck M, Wei X, et al. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut*. 2014;63:1960-1971.
27. Gremse F, Grouls C, Palmowski M, et al. Virtual elastic sphere processing enables reproducible quantification of vessel stenosis at CT and MR angiography. *Radiology*. 2011;260:709-717.
28. Snell GD. *Biology of the Laboratory Mouse*. Blakiston; 1941.
29. Willekens I, Buls N, Lahoutte T, et al. Evaluation of the radiation dose in micro-CT with optimization of the scan protocol. *Contrast Media Mol Imaging*. 2010;5:201-207.
30. Zhai J, He F, Wang J, Chen J, Tong L, Zhu G. Influence of radiation exposure pattern on the bone injury and osteoclastogenesis in a rat model. *International Journal of Molecular Medicine*. 2019;44:2265-2275.
31. Walter C, Grötz KA. Treatment with bone antiresorptive drugs – when should implantation take place? article in German. *Der junge Zahnarzt*. 2020;3:36-39.
32. Du Z, Lee RS, Hamlet S, Doan N, Ivanovski S, Xiao Y. Evaluation of the first maxillary molar post-extraction socket as a model for dental implant osseointegration research. *Clin Oral Implants Res*. 2016;27:1469-1478.
33. Aguirre JI, Altman MK, Vanegas SM, Franz SE, Bassit AC, Wronski TJ. Effects of alendronate on bone healing after tooth extraction in rats. *Oral Dis*. 2010;16:674-685.
34. Abtahi J, Agholme F, Sandberg O, Aspenberg P. Bisphosphonate-induced osteonecrosis of the jaw in a rat model arises first after the bone has become exposed. No primary necrosis in unexposed bone. *J Oral Pathol Med*. 2012;41:494-499.
35. Bamias A, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. *J Clin Oncol*. 2005;23:8580-8587.
36. Walter C, Al-Nawas B, Grotz KA, et al. Prevalence and risk factors of bisphosphonate-associated osteonecrosis of the jaw in prostate cancer patients with advanced disease treated with zoledronate. *Eur Urol*. 2008;54:1066-1072.
37. Giro G, Sakakura CE, Gonçalves D, Pereira RMR, Marcantonio E Jr, Orrico SRP. Effect of 17β-Estradiol and Alendronate on the removal torque of osseointegrated titanium implants in ovariectomized rats. *J Periodontol*. 2007;78:1316-1321.
38. Duarte PM, de Vasconcelos Gurgel BC, Sallum AW, Filho GR, Sallum EA. Alendronate therapy may be effective in the prevention of bone loss around titanium implants inserted in estrogen-deficient rats. *J Periodontol*. 2005;76:107-114.
39. Choi H, Lee JH, Lee JH, Kim JH. Genetic association between VEGF polymorphisms and BRONJ in the Korean population. *Oral Dis*. 2015;21:866-871.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Kniha K, Bock A, Peters F, et al. Microstructural volumetric analysis of the jaw following dental implantation under systemic bisphosphonate delivery: An in vivo and ex vivo rat study. *J Periodontol*. 2021;92:e66–e75. https://doi.org/10.1002/JPER.20-0547