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ORIGINAL ARTICLE

Microstructural volumetric analysis of vertical alveolar ridge augmentation using autogenous tooth roots

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Abstract

Background: To volumetrically assess the bone microstructure following vertical alveolar ridge augmentation using differently conditioned autogenous tooth roots (TR) and second-stage implant placement.

Materials and methods: The upper premolars were bilaterally extracted in n = 4 beagle dogs and randomly assigned to either autoclavation (TR-A) or no additional treatment (TR-C). Subsequently, TR were used as block grafts for vertical alveolar ridge augmentation in both lower quadrants. At 12 weeks, titanium implants were inserted and left to heal 3 weeks. Microcomputed tomography was used to quantify bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th), and trabecular spacing (Tb.Sp) at vestibular (v) and oral (o) aspects along the implant and in the augmented upper half of the implant, respectively.

Results: Median BV/TV [TR-C: 51.33% (v) and 70.42% (o) vs TR-A: 44.05% (v) and 64.46% (o)], Tb.th [TR-C: 0.22 mm (v) and 0.27 mm (o) vs TR-A: 0.23 mm (v) and 0.29 mm (o)] and Tb.Sp [TR-C: 0.26 mm (v) and 0.13 mm (o) vs TR-A: 0.29 μm (v) and 0.15 mm (o)] values were comparable in both groups.

Conclusion: Both TR-C and TR-A grafts were associated with a comparable bone microstructure within the grafted area.

KEYWORDS

alveolar ridge augmentation, animal experiment, micro CT, morphometry, tooth transplantation

1 | INTRODUCTION

Vertical alveolar ridge augmentation is considered to be an effective treatment approach for the rehabilitation of bone deficiencies in both partially and fully edentulous patients.[1] Autogenous bone (AB) blocks were frequently used for this specific indication and reported to result in a weighted mean vertical bone gain of 4.12 mm.[1] These outcomes, however, were associated with high complication rates (ie, 23%), mainly resulting in premature graft exposures.[1] Accordingly, it was recommended to perform implant placement in a staged approach.[2]

In a recent series of preclinical studies performed in a canine model, it was demonstrated that block grafts derived from autogenous tooth roots (TR) may serve as a feasible and effective alternative for lateral and vertical alveolar ridge augmentation. In particular,

histological and immunohistochemical analyses indicated that TR grafts were gradually replaced by newly formed bone and resulted in a significant horizontal and vertical bone gain.[3-6] Second stage implant placement was associated with a comparable formation of a new bone to implant contact (BIC) as noted at AB grafted sites.[3,5] To account for a potential contamination of grafts separated from potentially nonretainable, infected teeth, TR's were autoclaved and proven to exhibit a similar biological potential as nonautoclaved specimens.[6]

Recent microcomputed tomographic (μCT) analyses also focused on the assessment of bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th) and trabecular spacing (Tb.Sp), thus providing a volumetric analysis of TR following lateral ridge augmentation.[7,8] The segmentation of volumes of interest (VOIs) may also help to better understand the organization of TR associated with vertical grafting procedures. Therefore, the present study aimed at volumetrically assessing the bone microstructure following vertical alveolar ridge augmentation using differently conditioned TR's and second-stage implant placement in a standardized canine model.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental procedures

This study reports on a three-dimensional subset-analysis of tissue specimens derived from four beagle dogs, exhibiting a fully erupted permanent dentition (age 12.5 ± 0.6 months, mean weight 8.7 ± 3.9 kg). The histological assessments have been reported recently. In brief, the study was subdivided into three experimental phases, including defect creation (Phase 1), vertical augmentation of chronic-type defects at 12 weeks (Phase 2), and staged implant placement at 3 weeks (Phase 3).[6]

All animals were fed daily with soft-food diet and offered water ad libitum. The study protocol was approved by the Landesamt für Natur und Verbraucherschutz, Recklinghausen, Germany.

2.2 | Anesthesia protocol and experimental procedures

All surgical interventions were performed according to a standardized anesthesia protocol. In brief, 0.01 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany) was used for intramuscular sedation. Immediately after, anesthesia was initiated by using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). Inhalation anesthesia was accomplished by the use of oxygen, nitrous oxide and isoflurane. A constant rate infusion of lactated Ringer's solution was provided to maintain hydration during anesthesia. An intravenous injection of either 0.1 mg/kg piritramid (Dipidolor, Janssen-Cilag GmbH, Neuss, Germany) or 0.1 mg/kg l-methadon was used to improve the depth of anesthesia. Intraoperative analgesia was accomplished by injecting 4.5 mg/kg carprofene (Rimadyl, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative analgesia at days 1-3, 0.01 mg/kg buprenorphin was applied subcutaneously twice per day. During days 1-7, 4.5 mg/kg carprofen was provided per os.[6]

2.3 | Experimental phases

2.3.1 | Phase 1—Defect creation

Full flaps were raised in both hemimandibles and the premolars (PM2-4) as well as molars (M1-2) were carefully removed following tooth separation.

Two standardized box-type defects (6 mm in height from the crestal bone and 10 mm in width mesiodistally; distance between defects: 3 mm) were prepared using a carbide bur (n = 6 defects per animal). Flaps were repositioned and closed by means of mattress sutures (Resorba, Nürnberg, Germany) to allow for a submerged healing of 12 weeks.

2.3.2 | Phase 2—Vertical ridge augmentation

Two contralateral maxillary premolars (PM2) were removed after vertical tooth separation (n = 2 teeth) and the roots (n = 4) including the root pulp were separated at the cemento-enamel junction. Subsequently, TR's were randomly (RandList, DatInf GmbH, Tübingen, Germany) assigned to either a standardized autoclavation procedure (15 minutes, 134°C, 2.1 bar) (TR-A) or were left untreated (TR-C). Both sides of the mandible were augmented with 2 × TR-A and 2 × TR-C grafts, thus resulting in a total of n = 4 TR grafts per animal.

Following size adaptation of the coronal-apical extension to match the mesio-distal defect extension, TR's were positioned with either their mesial or distal interproximal surfaces facing the crestal alveolar bone. Dentin was exposed at the respective contact area by a careful removal of the cementum layer by means of a diamond burr under copious irrigation with sterile saline.[5] Subsequently, each specimen was fixed by one titanium osteosynthesis screw (P/5, 1.5 × 9 mm, Medicon, Tuttlingen, Germany; Figure 1A) and all sites were submerged by means of coronally advanced mucoperiosteal flaps and a combination of mattress and double sutures (Resorba).

2.3.3 | Phase 3—Implant placement

At 12 weeks following Phase 2, full flaps reflected to remove the osteosynthesis screws and perform an implant bed preparation and insertion according to the surgical protocol provided by the manufacturer. Commercially available screw-type titanium implants (⌀ 3.8 mm, length 7 mm, Conelog, Camlog Biotechnologies, Basel, Switzerland) were inserted in a way so that the implant shoulder (IS) at best coincided with the bone crest at the interproximal aspects (Figure 1B). Closure screws were applied and mucoperiosteal flaps repositioned to ensure a submerged healing condition for another 3 weeks.

2.4 | Retrieval of specimens

At the end of phase 3, the animals were euthanized by an overdose of sodium pentobarbital 3%. Dissected blocks containing the experimental sites were fixed in 10% neutral buffered formalin solution.

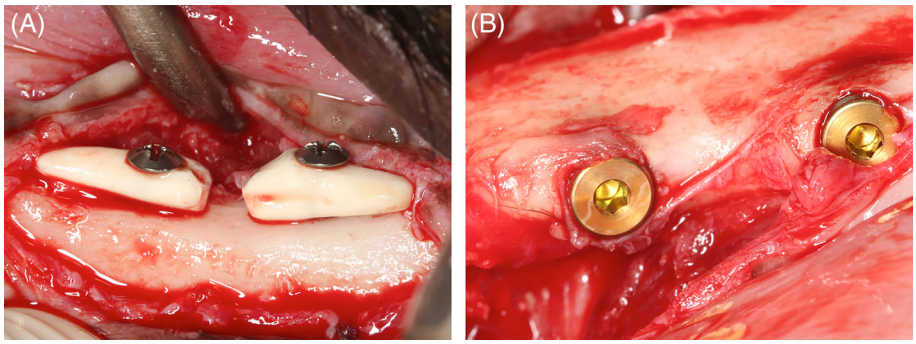

FIGURE 1 Surgical procedure. A, Adaptation and fixation of TR grafts for vertical augmentation at chronic-type alveolar ridge defects (Phase 2). B, Clinical situation following implant placement at re-entry after 12 weeks of submerged healing (Phase 3)

2.5 | Micro computed tomographic analysis

Tissue biopsies were dehydrated by means of ascending grades of alcohol and xylene, infiltrated and embedded in methylmethacrylate (MMA) (Technovit 9100 NEU; Heraeus Kulzer, Wehrheim, Germany). All MMA tissue blocks were scanned with a μCT device (Viva CT 80; Scanco Medical AG, Brüttisellen, Switzerland) at 70 kVp, 114 μA, 8 W, 31.9 mm FOV, an integration time of 1998 ms and 2× frame averaging. The data sets were reconstructed into 3D volumes with an isotropic nominal resolution of 15.6 μm voxel size.

2.6 | Image processing

Image processing was performed using the μCT Evaluation Software Program V6.5 (Scanco Medical AG, Brüttisellen, Switzerland). The method that was employed for image analysis has been described in detail previously.[8] In brief, all samples were re-oriented in a way that the implant axis corresponded to the *z*-axis by means of the *align in Z and reimport* script (Scanco Medical AG, Brüttisellen, Switzerland). Subsequently, a standardized cylindrical volume of interest (VOI) with a radius of 3.4 and 7.0 mm height was placed around the implant axis (maintaining a distance of 31.2 μm to the implant). Thereafter, the VOI was divided into two quarters, reflecting the buccal and oral aspects of each experimental site.

Segmentation of bone tissue within VOI enabled the assessment of morphometric indices and comparative analyses. Whenever a VOI encompassed elements outside the alveolar ridge, the respective subvolumes were manually removed from the VOI prior to the evaluations.

All evaluations were performed after application of a Gaussian filter (sigma = 0.8) and a threshold-based segmentation (22%). The following indices were assessed: BV/TV, Tb.Th and Tb.Sp which expresses the sizes of the marrow spaces. For all measurements, the guidelines for assessment of bone micro-structures using μCT were applied.[9] After assessment of the indices, the buccal and oral quarters were split in two equal height pieces along the implant axis and morphometric evaluation was repeated within the upper aspects, respectively.

2.7 | Statistical analysis

The statistical analysis was performed using the open-source software program R.[10] For each variable and group, data were pooled per animal and the respective medians and quartile ranges were computed. The Wilcoxon signed rank-test was used to perform within animal comparison between the test and control group, as well as between the buccal and oral aspect, respectively. Results were found significant at $P < .05$.

3 | RESULTS

3.1 | Volumetric analysis using micro CT

Visual examination pointed at a gradual replacement resorption. In 26% of the sites, the TR grafts were still detectable (TR-C: 18% of the sites, TR-A: 50% of the sites). Replacement resorption was most pronounced at the lateral aspects, where small trabeculae connected the graft with the pristine bone. Newly formed small trabeculae were also seen in vicinity to the implant and below the TR graft at the former defect bottom. In another 26%, small remnants of the root graft were detectable, and entirely surrounded by newly formed bone (TR-C: 27%, TR-A: 25%). Complete replacement of the graft was observed in the remaining specimen. In these cases, a dense network of highly connected mature trabeculae was seen in the augmented areas (Figures 2 and 3).

3.2 | BV/TV measurements

In the TR-C group, median BV/TV amounted to 51.33% (quartiles: 43.50%-59.59%) at the buccal and 66.74% (quartiles: 49.78%-70.80%) at the lingual aspect. In the TR-A group, this value was by trend lower at the buccal aspect (median: 44.05%, quartiles: 43.11%-44.98%, Wilcoxon $P = .8$) and comparable (median: 66.46%, quartiles: 59.47%-64.46%, $P = 1.0$) at the oral aspect. When comparing the BV/TV values between the oral and buccal aspect, a significant difference was noted ($P = .03$) (Figure 2).

In the upper half, median BV/TV amounted to 31.75% (quartiles: 24.83%-41.74%) at the buccal and 66.74% (quartiles: 58.40%-

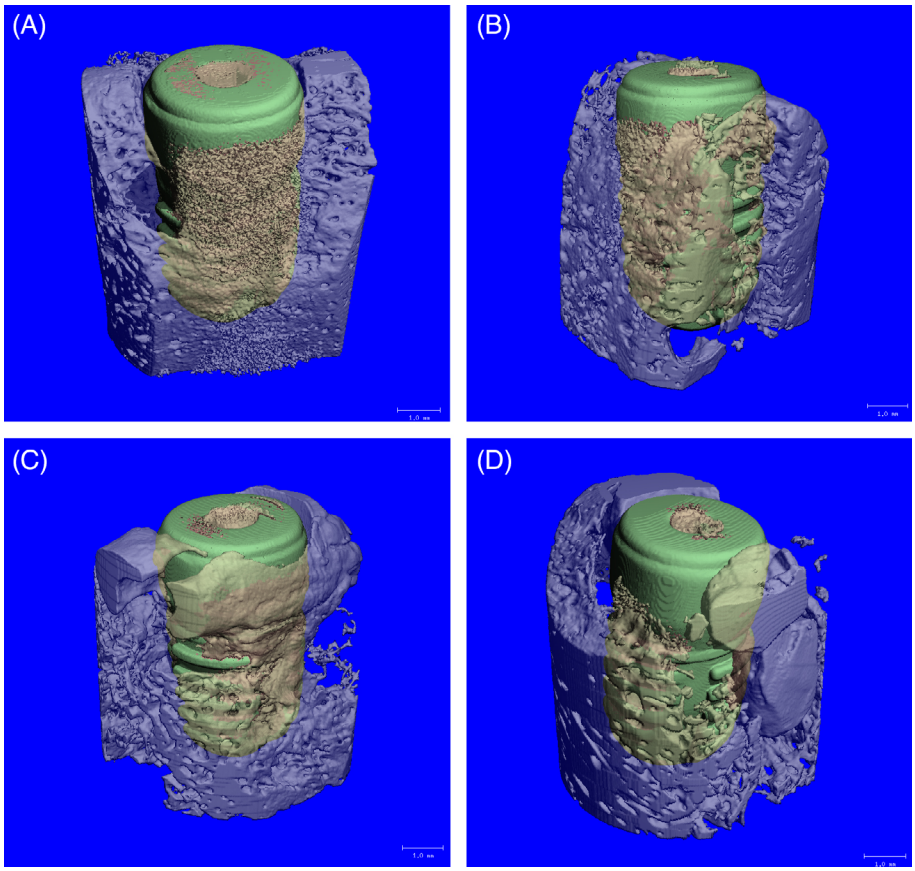

FIGURE 2 Volumetric analysis. A, Example showing a replaced graft of the TR-C group. At the former position of the graft, newly formed highly connected bone tissue can be seen. B, Example showing an entirely replaced graft of the TR-A group. At the former position of the graft, newly formed highly connected bone tissue can be seen. C, Example showing an incompletely replaced tooth root graft of the TR-C group. Patterns of replacement resorption are seen at the lateral and lower margins of the graft. D, Example showing an incompletely replaced tooth root graft of the TR-A group. Patterns of replacement resorption are seen at the lateral and lower margins of the graft

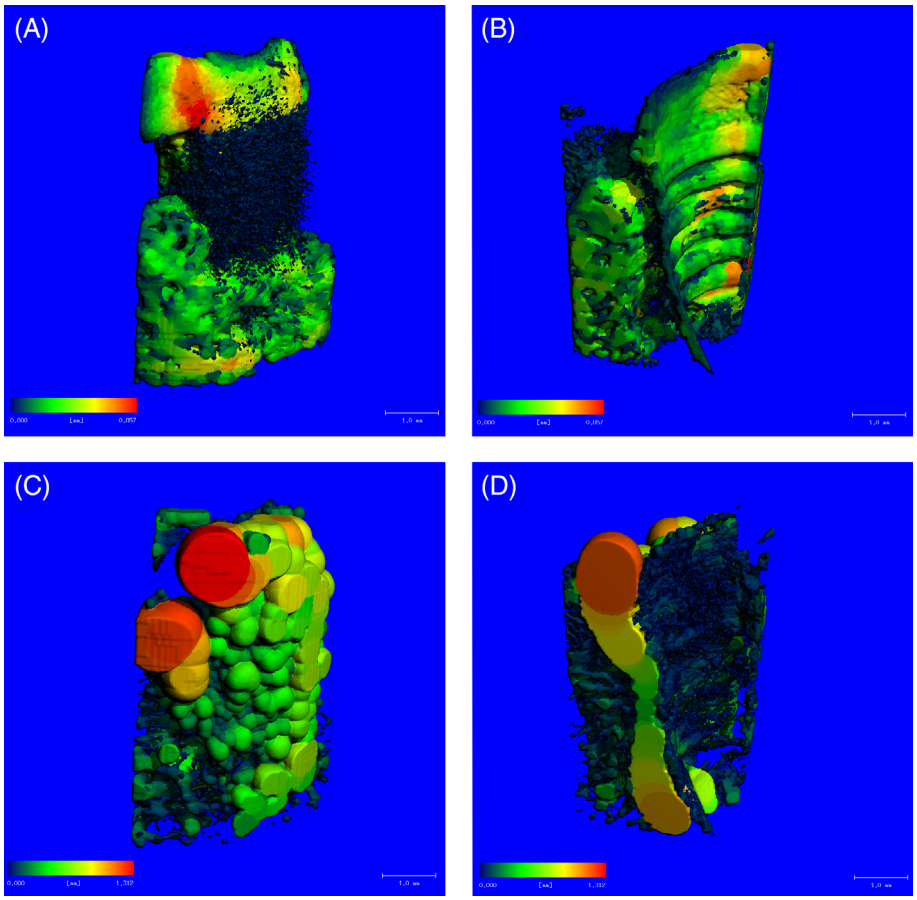

FIGURE 3 Assessment of Tb.Th and Tb.Sp. A, Example of Tb.Th values at the buccal aspect observed when a tooth root graft was still in place (see Figure 3C for the volume rendering). Thickness values are increased at the remnants of the tooth root graft and lower where newly formed woven bone has already been formed. B, Corresponding lingual aspect showing rather homogenous Tb.Th values. C, Example for the Tb.Sp values (buccal aspect) observed when a tooth root graft was still in place (see Figure 3C for the volume rendering). High values are observed in the void areas below the graft, whereas values reaching zero are seen at the localisation of the tooth root (mostly transparent). At positions where resorption already took place, small values colored in dark blue can be found

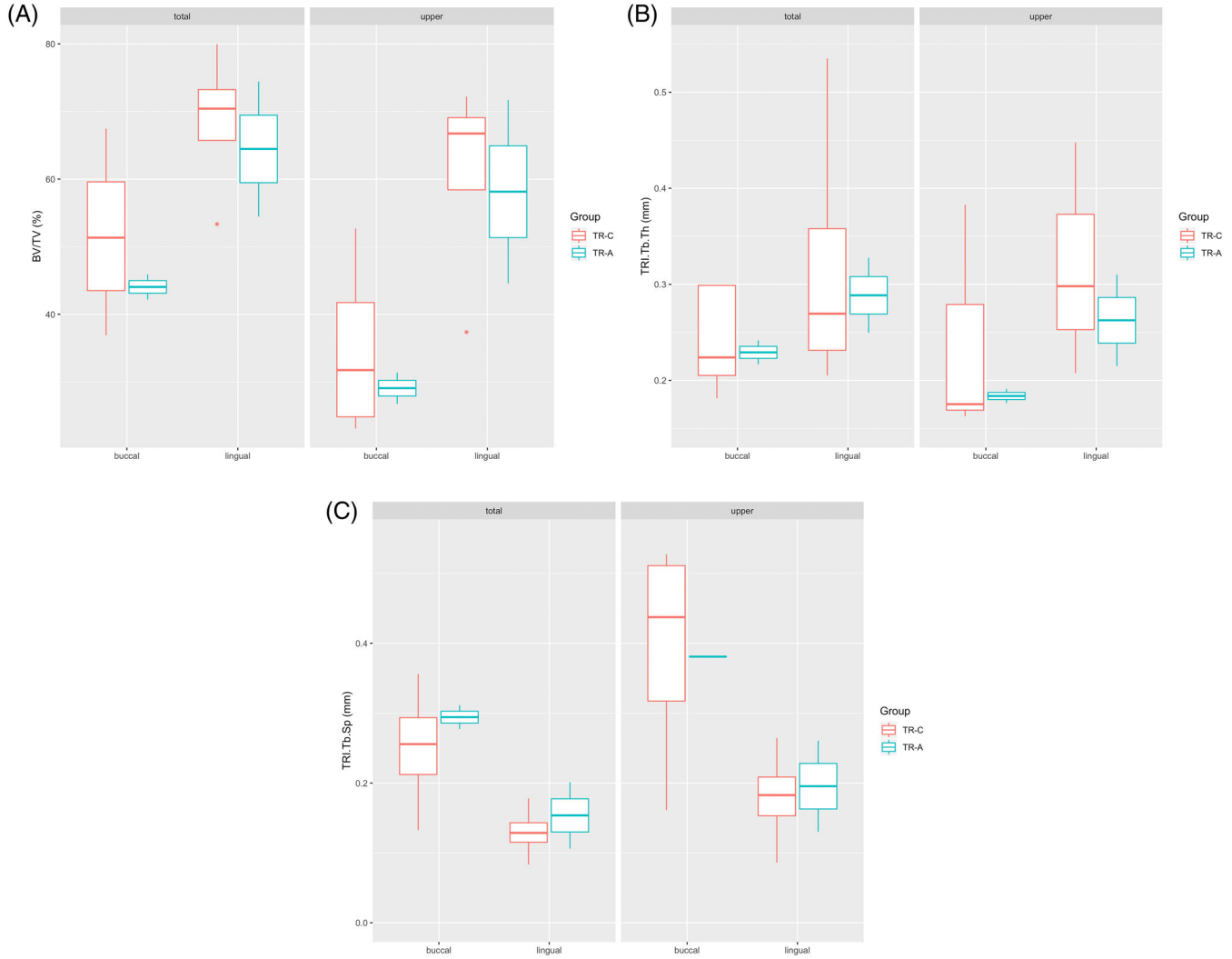

FIGURE 4 Boxplots for BV/TV, Tb.Th and Tb.Sp assessment. A, Boxplots showing the bone volume per tissue volume (BV/TV) at the buccal and oral aspects in the TR-C and TR-A groups (beside the entire implant and in the upper half). Significant differences were observed between the buccal and oral aspect ($P < .05$.) B, Boxplots showing the trabecular thickness values (Tb.Th) at the buccal and oral aspects in the TR-C and TR-A groups (beside the entire implant and in the upper half.) C, Boxplots showing the trabecular spacing values (Tb.Sp) at the buccal and oral aspects in the TR-C and TR-A groups (beside the entire implant and in the upper half). Significant differences were observed between the oral and buccal aspect, respectively ($P < .05$)

69.10%) at the lingual aspect in the TR-C and was by trend lower at the buccal (median: 29.08%, quartiles: 27.92%-30.23%, $P = 1.0$) and oral (median: 58.14%, quartiles: 51.35%-64.92%, $P = 1.0$) in the TR-A group. When comparing the BV/TV values between the upper oral and upper buccal aspect, a significant difference was noted ($P = .015$; Figure 4A).

3.3 | Tb.Th measurements

In the TR-C group, median Tb.Th amounted to 0.22 mm (quartiles: 0.21-0.34 mm) at the buccal and 0.29 mm (quartiles: 0.27-0.31 mm) at the lingual aspect. In the TR-A group, this value was comparable at the buccal aspect (median: 0.23, quartiles: 0.22-0.24 mm, Wilcoxon $P = .8$) and at the oral aspect (median: 0.29 mm, quartiles: 0.27-0.31 mm, $P = .8$). No significant differences were found between the buccal and oral aspect, respectively ($P = .24$; Figure 4B).

In the upper half, median Tb.Th amounted to 0.17 mm (quartiles: 0.15-0.23 mm) at the buccal and 0.18 mm (quartiles: 0.15-0.21 mm) at the lingual aspect in the TR-C and was by trend lower at the buccal (median: 0.29 mm, quartiles: 0.29-0.31 mm, $P = .53$) and oral (median: 0.27 mm, quartiles: 0.24-0.26 mm, $P = .8$) in the TR-A group. No significant differences were found between the upper buccal and upper oral aspect, respectively ($P = .18$; Figure 4B).

3.4 | Tb.Sp measurements

In the TR-C group, median Tb.Sp amounted to 0.26 mm (quartiles: 0.21-0.29 mm) at the buccal and 0.13 mm (quartiles: 0.12-0.14 mm)

at the lingual aspect. In the TR-A group, this value was comparable at the buccal aspect (median: 0.29, quartiles: 0.28-0.30 mm, Wilcoxon $P = .48$) and at the oral aspect (median: 0.15 mm, quartiles: 0.13-0.18 mm, $P = .8$). A significant difference was found between the buccal and oral aspect, respectively ($P < .001$; Figure 4C).

In the upper half, median Tb.Th amounted to 0.44 mm (quartiles: 0.32-0.51 mm) at the buccal and 0.48 mm (quartiles: 0.43-0.48 mm) at the lingual aspect in the TR-C and was by trend lower at the buccal (median: 0.48 mm, quartiles: 0.43-0.53 mm, $P = .37$) and oral (median: 0.19 mm, quartiles: 0.16-0.23 mm, $P = .8$) in the TR-A group. A significant difference was noted between the upper buccal and upper oral aspect, respectively ($P < .001$; Figure 4C).

4 | DISCUSSION

The present analysis aimed at volumetrically assessing the bone microstructure following vertical alveolar ridge augmentation using differently conditioned TR's and second-stage implant placement in a standardized canine model.

Basically, it was observed that both TR-A and TR-C groups were associated with comparable median BV/TV [TR-C: 51.33% (v) and 70.42% (o) vs TR-A: 44.05% (v) and 64.46% (o)], Tb.Th [TR-C: 0.22 mm (v) and 0.27 mm (o) vs TR-A: 0.23 mm (v) and 0.29 mm (o)] and Tb.Sp [TR-C: 0.26 mm (v) and 0.13 mm (o) vs TR-A: 0.29 μm (v) and 0.15 mm (o)] values, respectively. In this context, it must be emphasized that the image analysis has been conducted according to an established method[7,8] and that BV/TV, Tb.Th and Tb.Sp represent commonly used outcome measures for the volumetric μCT assessment of the trabecular bone microarchitecture adjacent to implant sites in jawbones.[9, 11, 12] Since the variability of these parameters is commonly impacted by local differences in the bone microstructure,[12-14] it is essential to define representative VOI's, which in turn may allow for the calculation of summative microstructural parameters for the different groups.[7,8] These differences may mainly be attributed to the rather heterogeneous replacement resorption associated with TR grafts, which has been noted after both later- and vertical alveolar ridge augmentation.[3,5,6]

When comparing volumetric μCT with histology, it must be noted that a high agreement has been reported for corresponding 2D sections.[12] Nonetheless, μCT and histology are complementary techniques. Whereas μCT enables analysis of the entire micromorphometric healing pattern including application of volumetric morphometric parameters,[9] undecalcified histological sections are restricted to two dimensions and a substantial information loss during processing. In contrast, only histology allows for cell-level analysis and immunohistochemistry at its specific cutting position.[7] For the present analysis, the goal was to extent the previous histological examination with a volumetric analysis, as histology did not reveal significant differences in bone morphometry.[6]

In particular, the μCT as well as the previous histological [TR-C: 7.28% (v) and 3.04% (o) vs TR-A: 4.55% (v) and 0.00% (o)] analysis of the present specimens ~~also~~ revealed that percentages of residual TR within the augmented area (AA) markedly differed between both groups. This was associated with distinct variations in respective AA values [TR-C: 0.64 mm^2 (v) and 2.36 mm^2 (o) vs TR-A: 0.22 mm^2 (v) and 2.36 mm^2 (o)], thus corroborating the present BV/TV analyses.[6] In this context, it must also be emphasized that implant placement at some of the sites investigated was associated with the occurrence of v-/o-dehiscence-type defects as the result of a narrow crestal ridge width. Accordingly, this may have impacted AA values and subsequently the present BV/TV analysis.

Similar BV/TV, Tb.Th and Tb.Sp values were also noted following lateral ridge augmentation using differently conditioned (ie, endodontically treated-E, periodontally infected-P) TR grafts. These values were within the range of those data noted for healthy TR and AB grafts.[8] Similar to the present analysis, Tb.Th values were also increased at residual TR, and decreased in the presence of newly formed bone. Following lateral ridge augmentation, it was noted that in all groups investigated, BV/TV, Tb.Th, and Tb.Sp values measured in the augmented v-VOI tended to gradually increase in the apical-crestal direction,[8] thus reflecting the pattern of bone formation which mainly originating from open marrow spaces at the defect bottom.[15,16]

The autoclavation procedure used for TR-A grafts was proven to be effective in "disinfecting/sterilizing extracted human teeth"[17] and was associated with a lower fracture resistance (40 minutes at 240 °F, 20 psi) when compared with nonautoclaved control teeth.[18] At least the volumetrical assessment of BV/TV, Tb.Th and Tb.Sp values after a healing period of 15 weeks did not reveal any marked differences in the microstructure of residual TR-A and TR-C grafts, thus reflecting that autoclavation had no major detrimental biological effect on TR specimens.

5 | CONCLUSION

Within the limits of the present analysis, it was noted that both TR-C and TR-A grafts were associated with a comparable bone microstructure within the grafted area.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests related to this study.

AUTHOR CONTRIBUTIONS

The authors contributions of the present research were the following: Frank Schwarz and Katrin Becker were involved in the concept-design, surgical procedures, data interpretation and writing/critical revision of the paper; Mira Kristin Hüfner, Manuel Nienkemper and Nicole Rauch were involved in data collection/analysis, data

interpretation, Puria Parvini was involved in data collection, data interpretation/analysis and paper drafting. All of the authors reviewed, approved the submission, and publication of the present manuscript.

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