

Architecture and Microstructure of Cortical Bone in Reconstructed Canine Mandibles After Bone Transport Distraction Osteogenesis

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Abstract Reconstruction of the canine mandible using bone transport distraction osteogenesis has been shown to be a suitable method for correcting segmental bone defects produced by cancer, gunshots, and trauma. Although the mechanical quality of the new regenerate cortical bone seems to be related to the mineralization process, several questions regarding the microstructural patterns of the new bony tissue remain unanswered. The purpose of this study was to quantify any microstructural differences that may exist between the regenerate and control cortical bone. Five adult American foxhound dogs underwent unilateral bone transport distraction of the mandible to repair bone defects of 30–35 mm. Animals were killed 12 weeks after the

beginning of the consolidation period. Fourteen cylindrical cortical samples were extracted from the superior, medial, and inferior aspects of the lingual and buccal plates of the reconstructed aspect of the mandible, and 21 specimens were collected similarly from the contralateral aspect of the mandible. Specimens were evaluated using histomorphometric and micro-computed tomographic techniques to compare their microstructure. Except for differences in haversian canal area, histomorphometric analyses suggested no statistical differences in microstructure between regenerate and control cortical bone. Morphological evaluation suggested a consistent level of anisotropy, possibly related to the distraction vector. After 12 weeks' consolidation, bone created during bone transport distraction osteogenesis was comparable to native bone in microstructure, architecture, and mechanical properties. It is proposed that, after enough time, the properties of the regenerate bone will be identical to that of native bone.

Opperman and Elsalanty are co-owners of the patent on the device and co-owners of Craniotech ACR Devices, LLC. All other authors have stated no conflict of interest.

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Introduction

The canine mandible is derived from the first visceral, or mandibular, arch; and through intramembranous ossification the dentary bone is formed [1]. This bone fuses with the condylar cartilage to create the body and ramus of the mandible. Before about 5 weeks of age, the rate of bone deposition is very rapid but then slows down; and by 7 months the mandible reaches a stable size, though bone remodeling continues [1]. Facial growth occurs in an imbalanced fashion, yet still maintains a functional equilibrium [2], mainly through deposition on the posterior

aspect of both the body and ramus, while being resorbed from the anterior surfaces [1]. Generally, the intramembranous formation of the craniofacial bones defines a particular orientation of the osteons within the mandible, which is associated with bone function, fracture repair, and mandibular treatments [3].

Using the principles of fracture repair for the treatment of deformities and defects of the mandible to restore its function, a new technique has gained momentum as a useful surgical procedure. The purpose of this procedure is not only to correct craniofacial genetic deformities, especially in cases of asymmetrical growth, but also to repair defects produced by cancer, gunshots, and trauma. Its first application was distraction osteogenesis (DO), a popular procedure used to generate new bony tissue between two edges by applying gradual tensile force. The procedure was first applied to the mandible by Snyder et al. [4] using a canine model, then more recently to correct human facial deformities [5]. A variation of the procedure is bone transport distraction osteogenesis (BTDO), in which a segment of bone is moved between two bony edges while regenerating a bone to bridge the gap (Fig. 1). BTDO was first applied in dog mandibles by Costantino et al. [6] and later in human patients [7]. Since BTDO has become widely used, its clinical results have been impressive not only in experimental animals but also in humans [8–11]. However, serious questions remain regarding how differences in biomechanical characteristics, architecture of haversian systems, and microstructure of the regenerated bony tissue when compared with the original bone would affect the restoration of mandibular function.

Although the biomechanical characteristics of the new bony tissue generated after DO have been addressed in different studies [12–15], only one previous study has focused on the mechanical properties of the new bone formed after BTDO [16], which showed a direct relationship

between mechanical properties of cortical bone and its microstructure, similar to Currey [17].

Panikarovskii et al. [18] first examined the histology of regenerate tissue formed through mandibular distraction, finding a relationship between the vector of distraction and the orientation of primary osteons. Subsequently, several experimental studies looked at the microstructure of the regenerated cortical bone after DO of the mandible. Most of them did this using histological evaluation [19–23] as well as a combination of both micro-computed tomography (μ -CT) and histological techniques [14, 24] to identify quantitative and qualitative microstructural characteristics of the newly formed tissue. However, no studies have performed either histological or μ -CT evaluation of bone tissues obtained after BTDO of the mandible. The objective of this work was to test the hypothesis that both microstructure and haversian structures are different in canine mandibular control bone compared to regenerated cortical bone obtained by BTDO. This difference might be a consequence of the differences in strain patterns during functional loads in the former and the tension strain during BTDO in the latter. Both histomorphometric and μ -CT techniques were used in this study.

Materials and Methods

Specimen Collection

In preparation for mandibular bone transport, a unilateral defect of 30–35 mm was created in the mandibles of five adult male American foxhound dogs. The jaw was then reconstructed with a novel bone transport reconstruction device (BTRP; Craniotech ACR Devices, Dallas, TX) composed of a reconstruction plate and an intraoral transport unit attached to a 10-mm bony disc (Fig. 1) [10]. The device

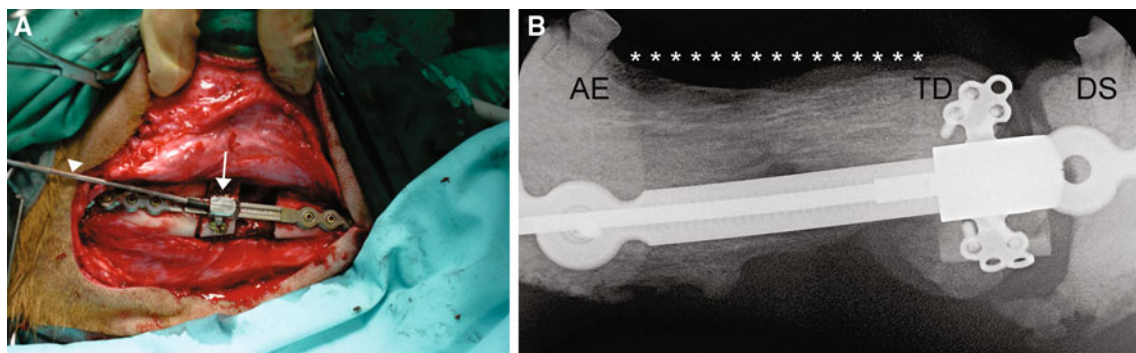


Fig. 1 **a** The mandibular bone transport (MBT) device in situ is positioned on the buccal side of the mandible. The transport segment is detached from the distal segment of bone, with the transport unit attached (*arrow*). The cable that protruded through the skin after wound closure activated the transport unit (*arrowhead*). **b** The MBT

device bridges the mandibular bony defect between the anterior edge (AE) and the docking site (DS). The transport disc (TD) was advanced from AE to DS, where the process is completed by compression stress. The new regenerate bone is clearly visible between AE and TD (*asterisks*), and it appears parallel to the direction of distraction

was activated 1 mm/day for 4–5 weeks, and then the new bone was allowed to consolidate for 6 weeks after the distraction was complete. After consolidation, dental implants were placed on both the experimental and control sides. The animals were allowed to heal for 6 more weeks before being killed and their mandibles dissected. Mandibles were stored at -20°C prior to removal of bone specimens to ensure that the bone's elastic properties were preserved [25]. A total of 35 cylindrical cortical bone specimens (average 5.93 ± 0.06 mm in diameter and 2.72 ± 0.24 mm in thickness) were removed from the lingual and buccal surfaces of the basal and alveolar regions of the mandible, from both the regenerate and control mandibular surfaces. Before removal, each sample was marked with a pencil line parallel to the base of the mandible to act as a visual marker of the sample's anatomical orientation. Twenty-one cylindrical cortical specimens were taken from the control side and 14 from the regenerate side using a low-speed dental drill continuously cooled with water during drilling. All sample specimens were stored in a 50:50 solution of 95% ethanol and isotonic saline at room temperature (19°C) in order to maintain the elastic properties of the bone [26]. Since only the properties of cortical bone can be assessed by ultrasound, all visible trabecular bone was carefully removed down to the cortical surface to avoid measuring trabecular spaces instead of cortical porosity. Cortical bone apparent density (mg/cm^3) was calculated from the sample weight using an analytical balance (PM460; Mettler-Toledo International, Columbus, OH) and differential volume in water, based on Archimedes' principle of buoyancy [26]. In addition, elastic mechanical properties were obtained for all specimens using an ultrasound test technique [16]. The direction of maximum strength was obtained with respect to the original reference line, and it was marked on the specimen by two notches that were clearly observed during three-dimensional $\mu\text{-CT}$ reconstructions (Fig. 2). After the mechanical properties were obtained for all specimens, the discs were scanned using $\mu\text{-CT}$ and subsequently embedded and sectioned for histomorphometric analysis.

$\mu\text{-CT}$ Analysis

$\mu\text{-CT}$ images were captured using a Scanco desktop cone-beam $\mu\text{-CT}$ 35 (Scanco Medical, Bassersdorf, Switzerland). To prepare the specimens for scanning, five 12.3-mm tubes were filled with a 50:50 solution of 95% ethanol and isotonic saline solution. The number of specimens placed in each tube varied from four to eight, with all samples in a single tube belonging to the same individual. The pencil mark parallel to the base of the mandible made before extraction was used to orient the specimens within the tubes. Before being scanned, specimens were viewed in scout view to verify their positions as well as to set the volume of interest to avoid extraneous

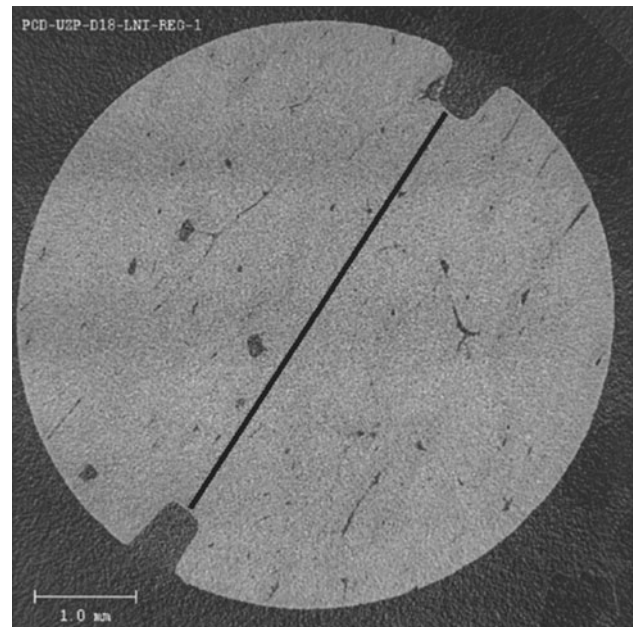


Fig. 2 Cylindrical regenerate cortical bone specimen reconstructed using $\mu\text{-CT}$. A marked continuous reference line matching with two notches represents the direction of maximum strength

scanning. Specimens were scanned at high resolution ($2,048 \times 2,048$ pixels) with a voxel size of $6 \mu\text{m}$ and a beam intensity of 70 KvP. An 800-ms integration time was used in order to reduce noise. After scanning, slices were contoured to specify a second volume of interest, which was the area to be analyzed for cortical porosity (Fig. 2). A logarithm typically used to characterize trabecular porosity was applied to cortical bone in order to measure cortical porosity. This made it possible to identify osteon orientation (Fig. 3). Thresholds were set to distinguish between gray-scale values for cortical bone and pore space. A lower threshold of $-1,000$ and an upper threshold of 307 were used, as well as a Gauss sigma of 1.5 and a Gauss support of 2 . The relative percentage bone volume (bone volume/total volume, Bv/Tv) and the degree of anisotropy (length of longest H-vector/shortest H-vector) were measured. The Bv/Tv is, in this case, a representation of the percentage of both haversian canals and voids within the cortical specimens. In addition, the degree of anisotropy is a representation of the structural conditions with the cylindrical specimen. A value close to 1.0 represents an isotropic characteristic, whereas values higher than 1.0 correspond to anisotropic conditions. The main advantage of $\mu\text{-CT}$ evaluation is that the specimens remain intact for the following histomorphometric assessment.

Histomorphometric Evaluation

Cylindrical specimens were split into two symmetrical parts through the notches used as a reference for the orientation of maximum strength and then fixed in 10%

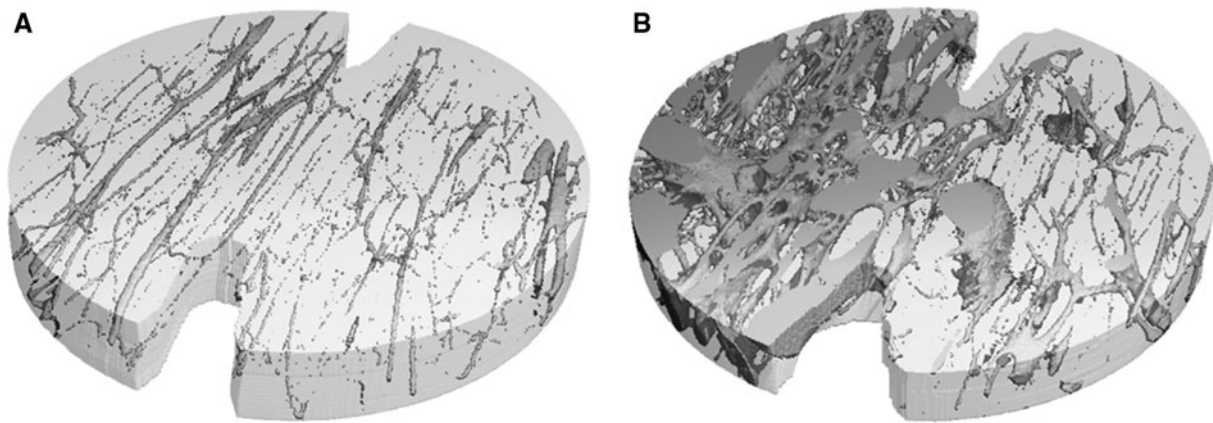


Fig. 3 μ -CT scans of cortical bone specimens on the control (a) and regenerate (b) sides. White, bone; gray, haversian spaces. U-shaped divots indicate the direction of maximal strength. Note that the haversian systems are orientated in the direction of maximal stiffness in both tissues

buffered formalin for 7–10 days. At the end of fixation, specimens were washed well in deionized water and then dehydrated, soaked in acetone for 2–3 days, and embedded in methyl methacrylate. Before being placed in the methyl methacrylate, four specimens at a time were put in a frame and covered in jade dental stone (Whip-Mix; Pearson Dental, Houston, TX) in order to embed multiple samples at once at the same orientation. Sections were cut using an Isomet low-speed precision sectioning saw (Buehler, Lake Bluff, IL) with a diamond blade.

Digital photographic registers of each specimen were recorded using a Nikon (Tokyo, Japan) Coolpix 4500 camera mounted on a Reichart-Jung (Nussloch, Germany) Microstar IV microscope via a microscope mount included with the camera. Pictures were taken in a panoramic sequence from superior to inferior then merged using Adobe Photoshop software (Adobe Systems, San Jose, CA) to create a composite image. These images were then transferred to a lab computer for analysis, and regions of interest were created within every specimen using BIOQUANT Osteo II software (Image Analysis, Nashville, TN), with areas ranging 0.622–0.638 mm². Only bony structures within this area were quantified. Haversian canal area, osteon area, and osteon number were measured and calculated using the histomorphometric software. Volkmann canals were counted manually and then recounted for accuracy. When an osteon was on the perimeter of the region, it was only measured if the haversian canal was on or inside the border. If the haversian canal was positioned outside of the borders of the region of interest, the osteon was not measured. Volkmann canals visibly associated with only one osteon and those acting as a connection between two osteons were counted as a single canal. Those Volkmann canals connecting three or more osteons were counted as separate canals for each osteon. Finally, the osteon number was divided by the area of interest in order to gain the osteon density per square millimeter.

Statistical Analysis

All analyses to test for differences between regenerated and control cortical bone among various regions of the mandibles and between subjects were done using SPSS statistical software (SPSS, Inc., Chicago, IL). One-way ANOVA was performed to test for histomorphological differences among the five subjects. In addition, independent sample tests were performed between 21 regenerate and 14 control specimens. Because cortical bone specimens were taken from consistent positions on the mandible at both the regenerated and control sites, *t*-tests were used to test for histomorphological differences between anatomical positions of the mandible (facial and lingual) by comparing five pairs of specimens from the regenerate bone obtained from three subjects against 10 pairs of control specimens obtained from five subjects and between relative positions of the mandible (alveolar and basal) by comparing six pairs of specimens from the regenerate tissue obtained from four subjects and nine pairs from the control bony tissue obtained from all subjects [16]. The statistical normality of the four variables considered in this study was tested using the Kolmogorov-Smirnov test, resulting in a positive normality for both osteon area ($P = 0.095$) and osteon number ($P = 0.20$), whereas the Volkmann canal number was slightly closer to the condition of normality ($P = 0.041$). Although haversian canal measurements did not reach the condition of normality ($P < 0.05$), it was assumed that ANOVA was robust enough to accommodate departures from this normality. In addition, a Levenes' test of homogeneity of variances was performed on the four variables; homogeneity was satisfied for osteon area ($P = 0.077$), osteon number ($P = 0.350$), and Volkmann canal number ($P = 0.492$) but not for haversian area ($P = 0.023$). No statistical evaluation was performed on the qualitative results obtained from the μ -CT process.

Results

Histological Examination

Photomicrographic observations suggested differences in the microstructure not only between the regenerate and control cortical bone (Figs. 4, 5) but also between buccal and lingual positions in the regenerate cortical bone (results not shown). In addition, it appears that the amount and size of the osteons were larger in the control than in the regenerated cortical bone, with fewer interstitial lamellae. In contrast to control bone, regenerate bone had smaller osteons with larger haversian canals and voids; under-mineralized interstitial lamellae and increased numbers of Volkmann canals were also noted.

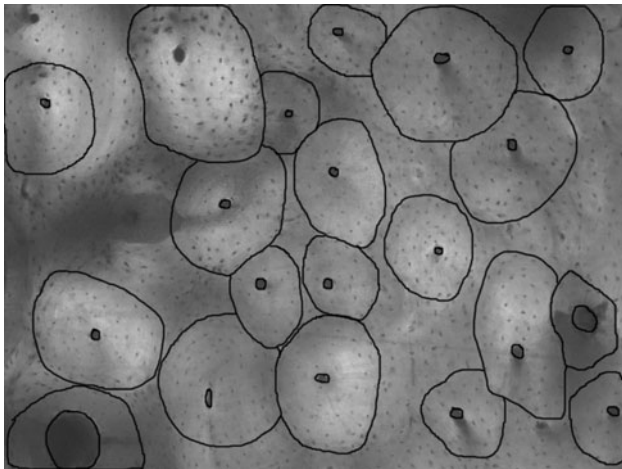
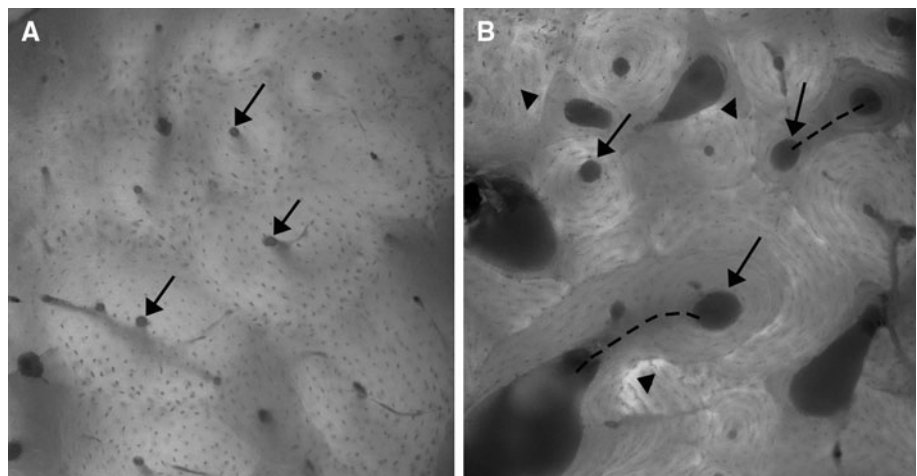


Fig. 4 Micrograph of a control cortical bone specimen showing how the quantitative histomorphometric evaluation of number of osteons, area of haversian canals (outlined in black), and osteon area (outlined in black) was done

Fig. 5 Histological sections from buccal control cortical bone (a) and buccal regenerated cortical bone (b). Note large haversian spaces in regenerate bone (arrows), less mineralized regions (arrowheads), and greater number of Volkmann canals (dotted lines) compared to control bone



μ -CT Analysis

Supporting the histological evaluation, μ -CT images showed that the lingual aspect of the regenerate cortical bone evinces more mature osteons of greater diameter than the buccal aspect (Fig. 3a, b). Whereas the lingual aspect of the regenerate more closely resembles the control, the buccal aspect of the regenerate contained fewer osteons but many osteocytes, usually associated with woven bone. However, we observed that the average osteon orientation in both control and regenerated cortical bone tended to follow the orientation of maximum strength (defined by the pair of notches), which was directly related to the mandibular basal plane for the control cortical bone (Fig. 3a, b) and the direction of the distraction vector for the regenerate cortical bone.

There was a statistical difference in the percentage of voids and haversian canals (Bv/Tv) between regenerate and control cortical bone ($F = 37.02$, $P < 0.02$). This was higher in the regenerate (14.9%) than in the control (2.2%) cortical bone (Fig. 6a). No statistical differences in Bv/Tv were present when comparing anatomical positions buccal–lingual or when comparing relative position basal–alveolar. There was a significant difference in the degree of anisotropy (DA) between regenerate and control specimens ($F = 0.06$, $P < 0.05$), which was slightly lower in the regenerate (1.72 ± 0.23) than in the control (2.05 ± 0.18) cortical bone (Fig. 6b). The pair test showed no significant differences in DA between anatomical positions buccal–lingual or between relative position basal–alveolar.

Haversian Canal Area

Independent sample t -tests showed no significant differences in haversian canal area between the regenerate and control cortical bone ($P = 0.062$, $F = 22.368$). The mean haversian canal area was $466.2 \pm 203.0 \mu\text{m}^2$ in control

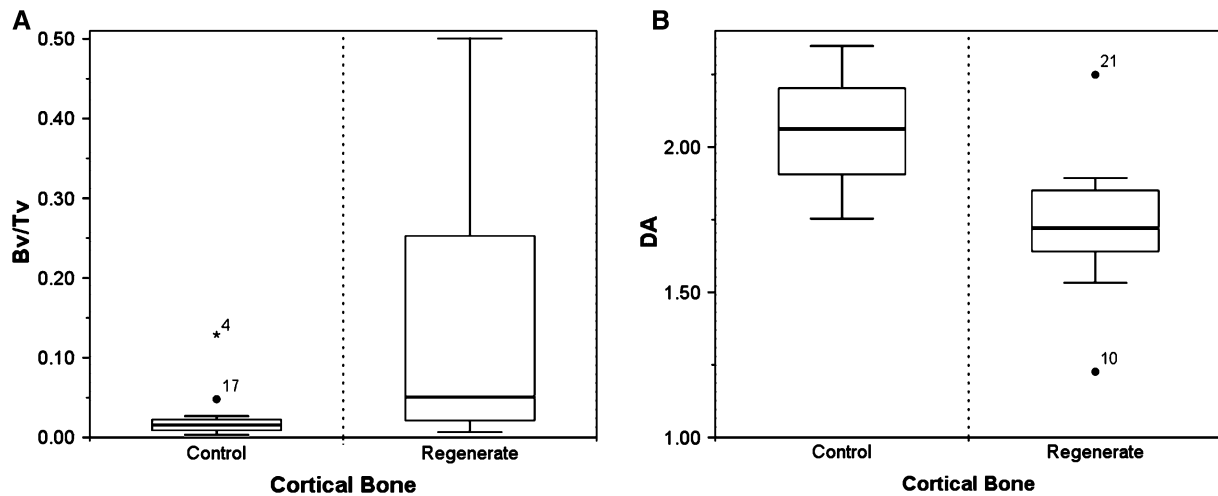


Fig. 6 Graphs showing μ -CT data for control and regenerate cortical bone. **a** Percentage of both voids and haversian canals (Bv/Tv). **b** Degree of anisotropy (DA), in which 1.0 indicates isotropic behavior

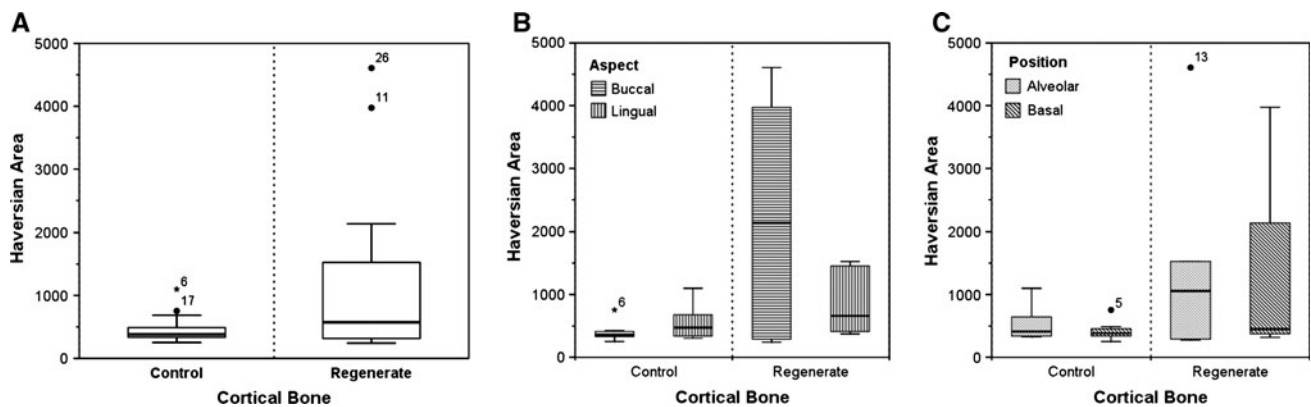


Fig. 7 Histomorphometric results for haversian canal area (μm^2) **a** between regenerate and control cortical bone, **b** between buccal and lingual aspects of the mandible, and **c** between alveolar and basal relative positions of the mandible

specimens and $1,244.0 \pm 1,417.5 \mu\text{m}^2$ in regenerate specimens (Fig. 7a). A paired *t*-test comparing the buccal and lingual specimens from the control sides found no significant differences, nor did a paired *t*-test comparing the regenerate buccal and lingual specimens (Fig. 7b). In addition, paired *t*-tests did not show statistical differences between both relative positions alveolar–basal for the control or regenerate cortical bone (Fig. 7c). When the 35 control and regenerate cortical bone specimens were combined, the mean haversian canal area for all specimens was $777.3 \pm 970.6 \mu\text{m}^2$.

Osteon Area

An independent samples *t*-test showed no significant differences in osteon area between regenerate and control cortical bone specimens (Fig. 8a). The mean osteon area for the regenerate cortical bone was $22,243.3 \pm 10,980.0 \mu\text{m}^2$,

whereas the mean for the control cortical bone was $20,691.6 \pm 3,796.9 \mu\text{m}^2$. Paired *t*-tests used to check for differences between buccal and lingual aspects showed no significant differences in control or regenerate specimens. Although the paired *t*-test showed significant differences ($P < 0.012$) between alveolar and basal bone of control specimens (Fig. 8c), there were no significant differences between these positions for the regenerate bone specimens. When combined, the mean for all 35 specimens was $21,312.2 \pm 7,427.8 \mu\text{m}^2$.

Osteon Density

There were no statistical differences in osteon density between regenerate and control cortical bone. The mean osteon density for the regenerate cortical bone was 26.5 ± 8.4 osteons/ mm^2 , whereas the mean for the control cortical bone was 28.3 ± 4.4 osteons/ mm^2 (Fig. 9a). Paired

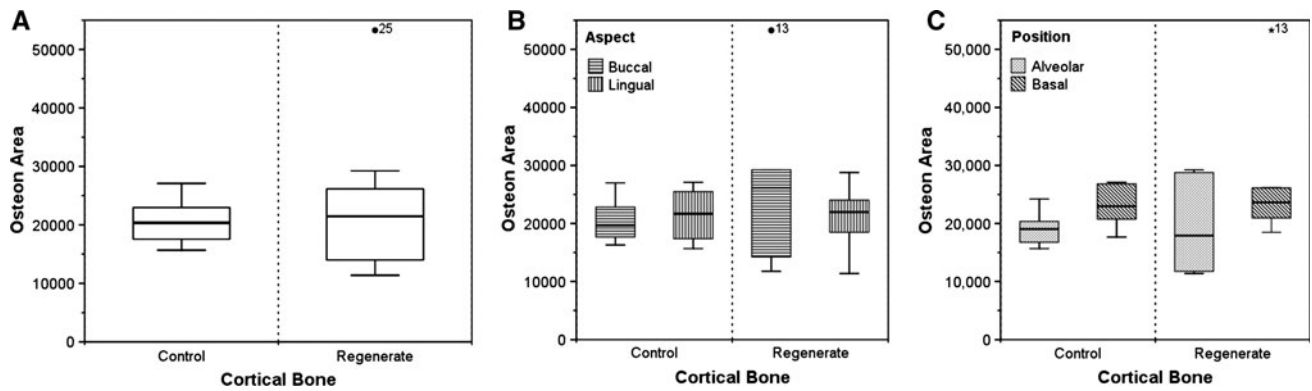


Fig. 8 Histomorphometric results for osteon area (μm^2) **a** between regenerate and control cortical bone, **b** between buccal and lingual aspects of the mandible, and **c** between alveolar and basal relative positions of the mandible

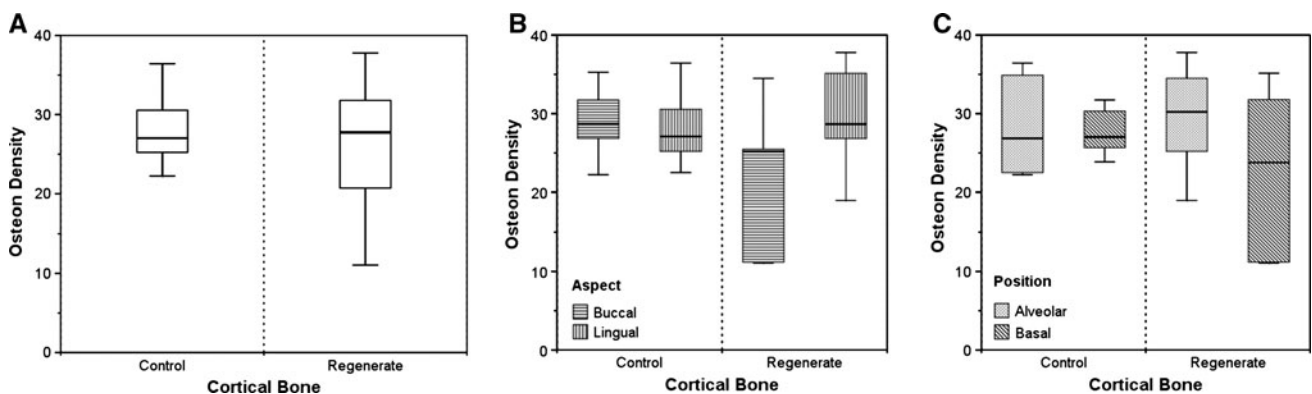


Fig. 9 Histomorphometric results for osteon density (μm^2) **a** between regenerate and control cortical bone, **b** between buccal and lingual aspects of the mandible, and **c** between alveolar and basal relative positions of the mandible

t-tests confirmed that there were no significant differences between buccal and lingual anatomical positions for the control or regenerate specimens (Fig. 9b). When the alveolar regions were compared with the basal regions, there were no significant differences within the control or regenerate cortical bone (Fig. 9c). When combined, the mean osteon density for all specimens was 27.6 ± 6.3 osteons/ mm^2 .

Volkman Canal Number

The independent sample test showed no statistical differences in Volkman canal number between the regenerate and control cortical bones. The mean of Volkman canal number for the regenerate was 7.0 ± 3.9 canals and for the control, 6.5 ± 2.9 canals (Fig. 10a). The paired *t*-test showed no significant differences between buccal and lingual anatomical positions within the control or regenerate cortical specimens (Fig. 10b). There were no statistical differences between alveolar and basal relative positions within control or regenerate cortical bone specimens (Fig. 10c). When combined, the mean for all specimens was 6.7 ± 3.3 Volkman canals.

Discussion

The objective of this work was to compare the microstructure and haversian structures of control bone compared to regenerated cortical bone obtained by BTDO in the canine mandible. The osteons in the regenerated cortical bone are oriented parallel to the bone transport vector of distraction, suggesting that the newly formed collagenous tissue mineralizes in that direction [22]. This finding is in concordance with the orientation of maximum material strength, implying a strong relationship between microstructure and mechanical properties [16]. These results are in agreement with those of Nomura et al. [3], who found that the osteonal orientation within a human mandible was parallel to the inferior border of the mandible and that the cortical bone of the mandible was transversely isotropic. Similarly, the osteonal structures within the control cortical bone were parallel to the basal aspect of the mandible and oriented with the direction of maximum stiffness of the material [16]. The osteon orientation of the regenerated cortical bone tends to be similar to the control cortical bone. Previous results have shown that control

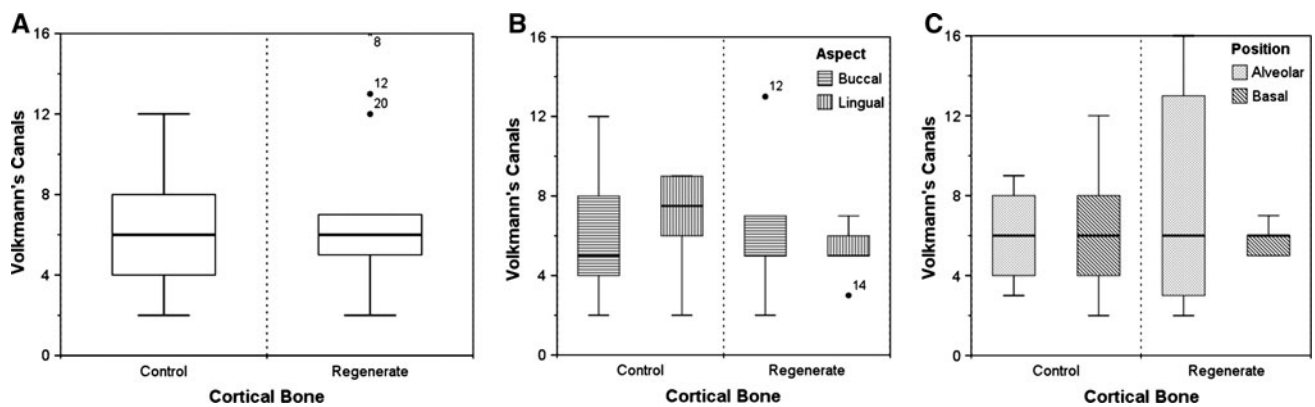


Fig. 10 Histomorphometric results for Volkmann canal number **a** between regenerate and control cortical bone, **b** between buccal and lingual aspects of the mandible, and **c** between alveolar and basal relative positions of the mandible

cortical bone tends to have orthotropic architecture, whereas regenerated cortical bone tends to be transversally isotropic, suggesting that the orientation of osteons within cortical bone may significantly influence its anisotropy [27]. Furthermore, osteon orientation in both control and regenerated cortical bone in the canine mandible contrasts with their longitudinal and helical distribution in human long bones as a consequence of the loading mode, a typical example of bone functional adaptation [28].

Both the degree of anisotropy and the orientation of osteonal structures indicate that the mineralized microstructure of the regenerated cortical bone tends to have the same orientation as the mechanical strain patterns produced within the new tissue by the traction vector of the BTDO device. The vector is parallel to the mandibular plane. This microstructural characteristic of the regenerate cortical bone is similar that of the original cortical bone of the mandible. These findings support the models of Cope and Samchukov [22] and Takano et al. [29], which claim a relationship between mechanical strain and mineral microarchitecture in bone tissue. If collagen microstructure of bone is influenced by mechanical strain [29], then the tension strain produced by BTDO devices would affect not only mineral microstructure of the new bone but also its elastic anisotropy.

According to Katz and Meunier [30], anisotropic elastic behavior of cortical bone depends on the microstructure of the osteons. In fact, several authors suggest that haversian cortical bone is transversely isotropic [31], whereas cortical lamellar bone is orthotropic. Much like Katz's work, a study conducted by Currey [32] indicates an association between haversian systems (secondary osteons) and cortical bone features in humans and some carnivores. Furthermore, μ -CT data in the current study suggest that the structure of the regenerate cortical bone tends to be anisotropic, either transversally isotropic or at least orthotropic as in the control cortical bone. This might be because the mechanical tensile strain produced by the BTDO device

is associated with the collagen microstructure of bone, thus increasing tissue anisotropy [29]. These results agree with previous work that reported anisotropic characteristics in both regenerated and control cortical bone after BTDO [16]. Interestingly, both maximum ultrasonic speed and the direction of maximum stiffness suggest that the microstructure of the regenerated cortical bone tends to be similar to the control cortical bone. This supports the argument that regenerate cortical bone created through distraction using internal BTRP devices tends to restore the mechanical characteristics, microstructure, and morphological features of the original mandibular bone.

A higher percentage of voids and haversian canals in the regenerate than in the control cortical bone appears to be an indicator of differing levels of mineralization between the two tissues. A qualitative inspection of the μ -CT results suggests the presence of more voids and fewer osteons in the regenerate bone. More voids and fewer osteons likely indicate a lower degree of maturation of this cortical bone [33]. Some cartilage, fibrous tissue, and woven bone associated with voids were found in the regenerate samples. This is to be expected when the callus outgrows its vascular supply in order to bridge the gap with new bony tissue. Although cartilage and soft tissues may contribute to reduced stability during the BTDO process, a gradual decrease of the soft tissue with increasing consolidation time is expected. This expectation is in agreement with Cope and Samchukov [22], who showed not only a decrease of the fibrous tissue over the consolidation period but also the presence of remodeling after the eighth consolidation week. Also, histomorphometric results from Sencimen et al. [20] showed that the quantity of newly formed bone increased with increasing consolidation time. In addition, it is important to consider the effect that chewing forces could have on bone maturation [20].

Histomorphometric results in the haversian canal area between regenerate and control cortical bone agree with

those of Sencimen et al. [20], who did not observe histological differences between the newly formed bone and native bone. The larger haversian canals seen in the regenerate compared to control cortical bone suggest that mineralization of the regenerate is still in process. In addition, the difference in osteon area between basal and alveolar positions in the control cortical bone, which is not present in the regenerate specimens, suggests different biomechanical tissue responses to different physiological conditions. This difference could be associated with bending of the bone in the part of the mandible that is not supported by the BTRP device. Although we did not find any statistical differences between the buccal and lingual regions of the regenerate and control cortical bone, Mulder et al. [33] reported differences in both architecture and degree of mineralization between buccal and lingual plates of the pig mandible, characterized by a more compact structure and higher mineralization in the lingual region. Their findings agree with previous work that reported differences in mechanical properties between buccal and lingual positions of the canine mandible in the regenerate cortical bone, which were assumed to be associated with the presence of the device on one of the sides of the mandible [16]. The device was placed on the buccal side of the regenerate, so these tests were performed to determine if the device had any effect on the microstructure of the bone. Although these biomechanical differences can be associated with the fact that the maturation of the cortical bone may alter the mechanical properties at the microstructural level [34], it is also possibly associated with differences in the bone formation process.

There are limitations to the present study. For example, the animals were drawn from a large sample of dogs used to study different consolidation periods of BTDO; thus, five animals were considered in our study. This reduction in the number of animals limited the statistical power, although the number of cortical bone specimens used for comparisons is appropriate. Specimens used as control bone are obtained from the same animal by removing them from the side contralateral to the device. Thus, increased physiological loads on the control side during the 17 weeks of the distraction process could affect control bone properties. These options were chosen to reduce the number of animals required for the study. In spite of this approach, and in contrast to the hypothesis, there were no differences in microstructure between regenerate and control cortical bone, except for the difference in density that suggests an incomplete mineralization of the new tissue. Given a longer consolidation period, it is probable that the regenerate cortical bone would eventually become comparable to the native bone. Finally, these results appear to support the argument that BTDO is able to successfully generate new cortical bone similar to the original bone. In addition,

BTDO restored the mechanical characteristics, microstructure, and morphological features of the original cortical bone in the mandible, which facilitate the restitution of mandibular function.

Conclusion

The combined use of histology to characterize the maturity of the new cortical bone when compared with control bone and μ -CT to compare the anisotropic differences between regenerated and control tissues was helpful in understanding how the strain patterns generated in the mandible by BTDO affect microstructure, architecture, and mechanical properties of cortical bone. There is an obvious orientation of the basic haversian system parallel to the vector of distraction in the regenerate cortical bone, and the same pattern is present in the control cortical bone but with their orientation parallel to the base of the mandible. This similarity suggests no significant differences in microstructure between the new regenerate and control cortical bone, except for the level of mineralization reached at this specific consolidation period. Furthermore, it is important to note the effect of the vector of distraction on the patterns of microstructure associated with the regenerate tissue.

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