Bone Response to Functioning Implants in Dog Mandibular Alveolar Ridges Augmented with Distraction Osteogenesis

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The specific aim of this study was to determine the response of alveolar bone after it was augmented vertically using distraction osteogenesis and subsequently loaded with implant restorations. Four dogs each had four implants placed horizontally into an edentulous mandibular quadrant and, after integration, a distraction osteogenesis device was fabricated in the laboratory. An osteotomy was made to allow the crest of the alveolar ridge to be distracted vertically. After 10 mm of vertical distraction, the device was stabilized with light cured resin. Following bone fill confirmation of the distraction gap at 10 weeks, two implants were placed into the ridges, one in distracted bone and one in nondistracted bone. After 4 months for implant integration, freestanding prostheses were fabricated. Crestal bone levels were evaluated throughout the period of function. Animals were sacrificed after 1 year of loading, for histologic evaluation of the bone. The vertical ridge augmentation averaged 8.85 ± 1.05 mm after 10 weeks of healing following distraction, without change over 1 year of implant loading. Histologic examination showed that bone had formed between the distracted segments, creating an augmented ridge. The average thickness of the labial cortex in the distraction gap was significantly thinner than the lingual cortex in distracted bone and the lingual and labial nondistracted cortical bone. The presence of the dental implant did not significantly affect cortical bone thickness. Serial sections showed that implants remained integrated and functional without soft tissue inflammation. Dental implants placed into alveolar ridges augmented with the technique of distraction osteogenesis maintained bone and were functional for the length of this study.
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Key words: alveolar ridge augmentation, distraction osteogenesis, implant restorations

Patients with inadequate mandibular bone height and width do not qualify for placement of dental implants unless the deficient areas have bone grafts placed or the inferior alveolar nerve repositioned. These techniques have morbidity associated with them, such as pain from the donor site and paresthesia from nerve manipulation. A previous study[1] indicated the possibility of using distraction osteogenesis for augmenting the alveolar ridge. The present study used the same animal model to examine the loaded bone response to dental implants placed into alveolar ridges augmented with distraction osteogenesis after 1 year of function.

Distraction osteogenesis is a technique that has been applied to lengthen or repair continuity defects in the mandible, maxilla, and cranial complex.[2–24] llizarov[23] has shown that distraction of bone in a transverse vector to a bone's long axis can result in bone formation and stabilization of the distracted bone. The same phenomenon has been demonstrated in dogs by distracting the zygomatic process of the dog laterally.[24] One consistent observation is

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that soft tissue neogenesis accompanies the hard tissue production. The hypothesis is that by distracting the superior surface of the alveolar ridge, sufficient bone and soft tissue can be generated to allow for dental implant placement and functional rehabilitation of the atrophic ridge.

Materials and Methods

Surgical Procedures. Four heartworm-free mongrel dogs were placed under pentobarbital intravenous general anesthesia, their left mandibular premolars and first molars were extracted, an alveoloplasty was performed to simulate an atrophic ridge, and the opposing maxillary teeth also were extracted to prevent ridge trauma during chewing. After 12 weeks of healing, a crestal incision was made and the mucosa was reflected, exposing the lateral surface of the mandible. Four 8-mm-long, 3.25-mm-diameter hydroxyapatite-coated cylindrical implants (Sulzer Calicitek, Carlsbad, CA) were placed horizontally through the buccal cortex, engaging the lingual cortex. The implants were placed 20 mm apart and with a vertical distance of 10 mm between them. Healing screws were placed, and 10 weeks were allowed for implant integration.[1] The dogs received 2 million units of procaine penicillin for 5 days and chewed a softened diet until they were restored: they then ate a normal textured dog chow, and were observed daily for adequate food intake.

After 10 weeks for implant integration, the dogs were reanesthetized, a crestal incision was made, and the lateral aspect of the mandible was again exposed after subperiosteal reflection. Five-millimeter shouldered abutments were placed into the horizontally oriented implants, and transfer impressions were made. The healing screws were then replaced, and the incision was closed. In the laboratory, a device used for palatal expansion was oriented vertically and waxed to plastic waxing sleeves placed on the abutment analogues. The four distraction devices were cast as one unit.[1]

The dogs were again reanesthetized, a crestal incision was made, and a supraperiosteal dissection was done, preserving the periosteum over the lateral surface of the mandible. The healing screws were removed, and the abutments were replaced. The distraction device was seated to confirm a passive fit. A thin fissure bur was then used to create an osteotomy between the superior- and inferior-positioned implants, extending vertically posterior and anterior to the implants, creating a mobile piece of alveolar bone that contained the two superiorly placed implants (Fig 1). After the osteotomy was completed, the distraction device was secured to the implant abutments with screws, and the incisions were closed (Fig 2). Holes were made through the lateral mucosa to allow the abutments to protrude, since a crestal incision and a vestibular-based flap had been used for access. The distraction device was not activated for 7 days to allow for periosteal healing and early revascularization.

After 7 days, the mandible was distracted superiorly 0.5 mm twice a day for 10 consecutive days (Fig 3). The dogs were gently restrained and showed no discomfort during the distraction process. On the tenth day, radiographs were taken with the dogs under general anesthesia, and the device was stabilized with light-cured resin.

After 10 weeks were allowed for bone healing within the distraction gap, two 16-mm dental implants (Mark II, Nobel Biocare USA, Westmont, IL) were placed. This length was chosen because the distraction gap was 10 mm and the thickness of the distracted segment of bone was approximately 6 to 8 mm in height. One implant was placed in the distracted ridge, and the second was placed in nondistracted bone posterior to the distraction in the first molar region (Fig 4). Four months were allowed for integration. At this time, transfer impressions were made, and freestanding prostheses to be supported by the two implants were fabricated using precious alloy. These were then placed and screw retained (Fig 5). Lateral radiographs were taken 1, 2, 6, and 12 months after prosthesis placement (Figs 6a to 6f). The dogs were sacrificed 1 year after delivery of the prostheses. At sacrifice, the mandibles were retrieved intact and fixed in 10% neutral buffered formalin for 10 days. The mandibles were sectioned and radiographed (Fig 6), and then processed for histologic evaluation.

Clinical Evaluation. The screw holes in the abutments were used as measuring points to determine the vertical distance between the implants in both the anterior and posterior positions. These distances were recorded prior to the osteotomy, after 10 days of distraction, and after 10 weeks of healing. The soft tissues directly over the distraction site were examined for signs of inflammation or breakdown during all phases of the study, and qualitative evaluations of this health were recorded.

Radiographic Evaluation. Radiographs were taken, with the film placed parallel to the lingual cortex, immediately after the osteotomy, after the tenth day of distraction, 10 weeks after distraction was completed, after implant placement, immediately after prosthesis placement (baseline value), and at 1, 2, 6, and 12 months after prosthesis placement. These films were used for qualitative evaluation of bone healing in the distraction gap and to measure crestal bone levels during implant loading. No bone markers

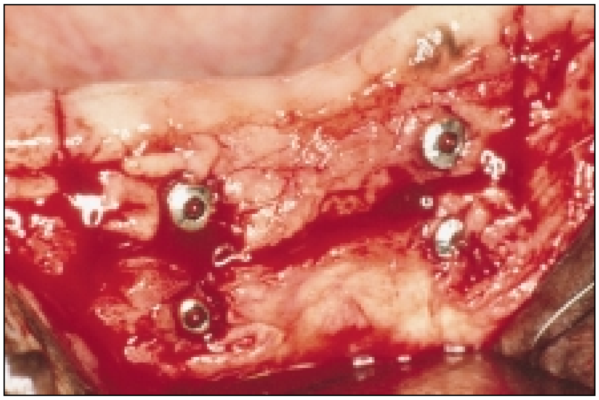


Fig 1 View of osteotomy site. Four hydroxyapatite-coated, 3.25-mm-diameter, 8-mm-long implants were placed horizontally to serve as anchors for the distraction device. After the implants integrated, impressions were made, and a distraction device was fabricated in the laboratory. Here, a supraperiosteal dissection exposed the lateral surface of the mandible, and the osteotomy was performed full thickness with a thin bur.

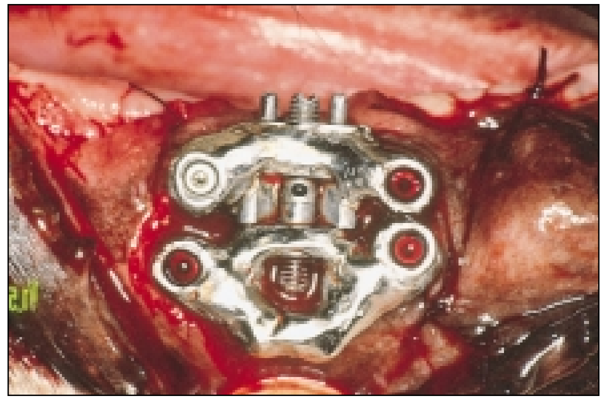


Fig 2 After completion of the osteotomy, abutments were placed into the implants, the distraction device was secured to the abutments with screws, and the soft tissues were closed.

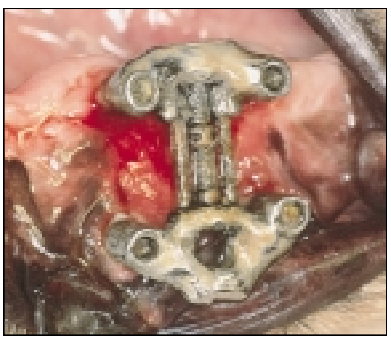


Fig 3 The ridge was distracted 0.5 mm two times per day for 10 consecutive days.

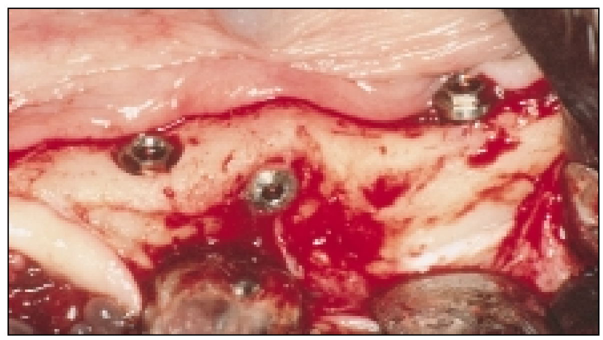


Fig 4 After 10 weeks were allowed for bone formation within the distraction gap, two implants were placed, one into the distracted ridge and one into nondistracted bone.

were placed on the crest of the superior segment to avoid excessive tissue reflection and the potential decrease in vascularization of the segments; therefore, no measurement reference point was available to determine the stability of the thickness of the distracted alveolar bone. The lateral radiographs were superimposed to evaluate stability of the implant-implant distance of the horizontally placed implants that had been used to anchor the distraction device.

Histologic Evaluation. After sacrifice by intracardiac pentobarbital 52 weeks following prosthesis placement, the mandible was removed en bloc. Following 10 days of fixation, the prostheses were removed, and the mandibles were radiographed. The mandibles were sectioned from facial to lingual to form cross sections of bone that included non-

Fig 5 After 4 months were allowed for implant integration, a fixed prosthesis was fabricated and secured to the implants with screws.

Figs 6a to 6f Serial radiographs of the distraction site.

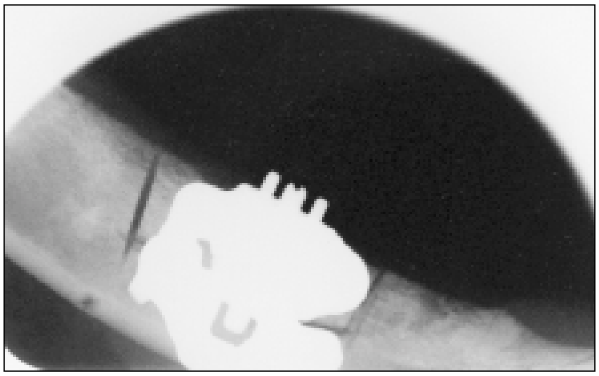


Fig 6a Lateral radiograph taken immediately after the osteotomy was performed and the distraction device placed.

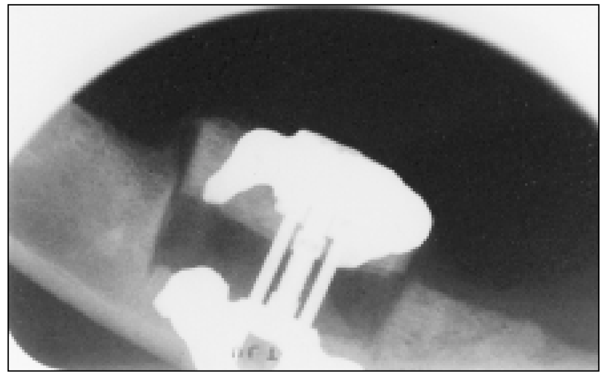


Fig 6b Lateral radiograph taken after 10 days of distraction.

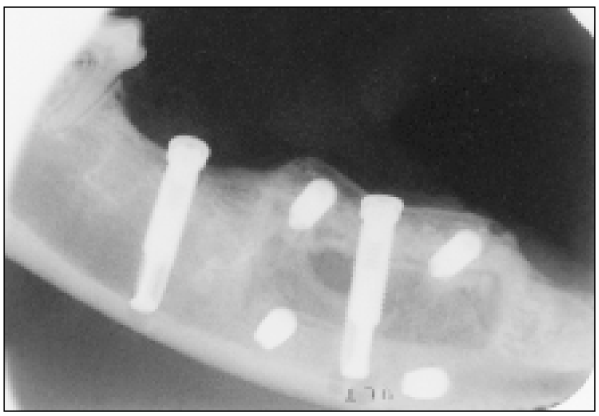


Fig 6c Lateral radiograph taken after 10 weeks of healing, after placement of the implants used to support the freestanding prosthesis.

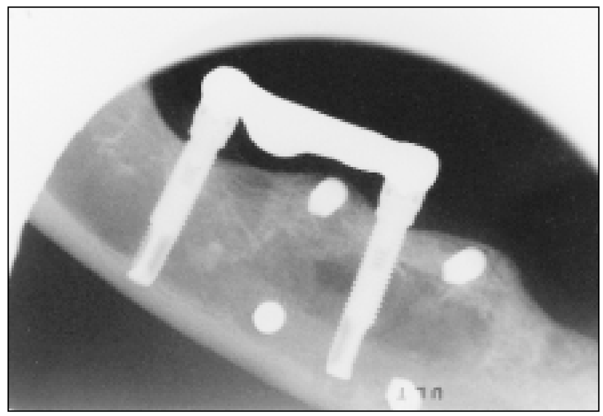


Fig 6d Lateral radiograph taken immediately after placement of prosthesis.

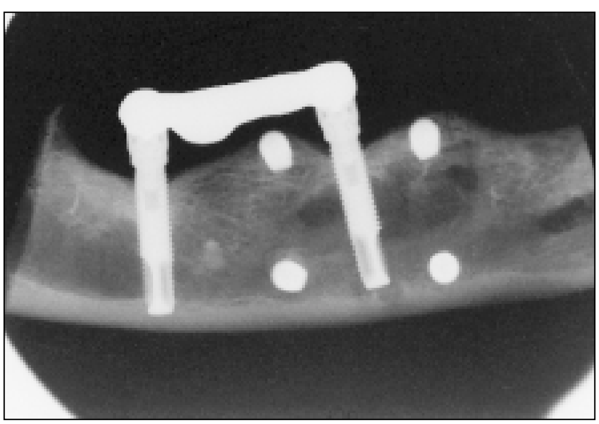


Fig 6e Lateral radiograph taken after 1 year of loading. Note bone remodeling and crestal bone levels adjacent to the implants.

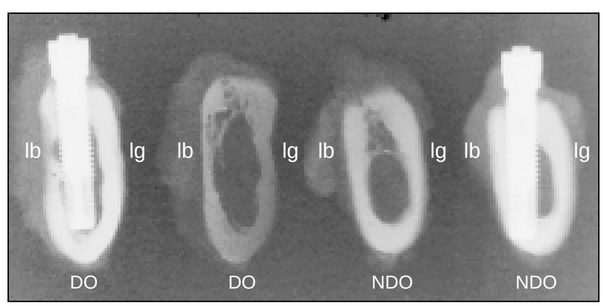


Fig 6f Cross-section radiographs of the specimens taken prior to histologic processing. Note the difference in ridge height for the distracted bone (DO) and the nondistracted (NDO) bone. Note also the thickness of the labial (lb) and lingual (lg) cortical bone and that the labial bone on the distracted ridges is thinner than the lingual cortex.

Table 1 Crestal Bone Levels of Implants Placed into Distracted and Nondistracted Alveolar Ridges

Ridge treatment	Time period	N	Mean	SD
Distracted	Baseline	4	1.51	0.60
	1 month	4	0.06*	0.05
	2 months	4	0.30*	0.20
	6 months	4	0.66*	0.09
	12 months	4	0.77*	0.29
Nondistracted	Baseline	3	2.27	0.57
	1 month	3	0.18*	0.07
	2 months	3	0.29*	0.34
	6 months	1	0.90*	—
	12 months	3	0.51*	0.24

Bone level was measured from top of shoulder of implant (abutment interface) to first bone contact with implant.
*Represents the difference from the baseline value; the positive value indicates loss of crestal bone.

distracted bone with and without implants and distracted bone with and without implants. After radiography, these cross sections were embedded in resin. Undecalcified serial sections were cut at 30 µm using a Leitz microtome (Reichert-Jung, Freiberg, Germany) and stained with Alizarin red. Because of the differences in initial ridge width between animals and the limited number of implants used, the amount of bone in direct contact with the implants was not calculated.

Using the Olympus Cue-2 image analysis system (Olympus, New York, NY), the thickness of the labial and lingual cortical bone within the distraction gap and in similar areas in nondistracted bone, with and without implants, was recorded. The inferior alveolar nerve was used as a reference point to locate similar areas of cortex for the nondistracted and distracted bone specimens. The thickness of the cortical bone formed was compared to nondistracted bone using analysis of variance (ANOVA).

Results

Clinical Evaluation. The dogs tolerated the surgical procedures well. During the osteotomy, the inferior alveolar artery was severed in one animal, without obvious clinical sequela. The soft tissues healed well without evidence of infection or breakdown. The mucosa over the distraction site was normal in appearance. The tissue within the distraction gap felt bone hard at 6 weeks, which was consistent with the radiographs.

The vertical distance between the implants, as measured from the center of the abutments, averaged 9.05 ± 1.01 mm after the initial 10 days of distraction, and 8.85 ± 1.05 mm after 10 weeks of healing. Superimposition of lateral radiographs demonstrated that there were no changes in the distance between the horizontally placed dental implants used to anchor the distraction device stability after 1, 2, 6, and 12 months of prosthesis function.

At the time of sacrifice, the tissue along the lingual and facial cortices was bone hard with healthy-looking soft tissue along the ridge and around the implants. The soft tissue that had been created through the distraction process was intact and appeared similar to baseline time period observations (Fig 5).

Radiographic Evaluation. The distraction defect was easily distinguished throughout the time period evaluated in this study (Fig 6). The anterior and posterior edges of the segments appeared to heal with mineralized tissue by the sixth week. From 6 to 10 weeks, and until sacrifice at 12 months after loading, the radiographic density of the bone between the distracted segment and the remaining corpus of the mandible increased. Radiographs of the cross sections indicated a similar morphology between nondistracted and distracted specimens. The labial cortex of the distraction regions had areas that were thinner than the lingual cortex and both cortices of nondistracted bone, and also areas that were similar without obvious thinning of the cortices.

The crestal bone levels as measured from the abutment-implant connection are found in Table 1. Means and standard deviations were calculated for the radiographic measurements of the implants for the distracted and nondistracted sites. The means were calculated for each time period (baseline, 1 month, 2 months, 6 months, and 12 months). The values for the mesial and distal measurements were averaged for each dental implant. Differences from the baseline measurements were calculated for each time period.

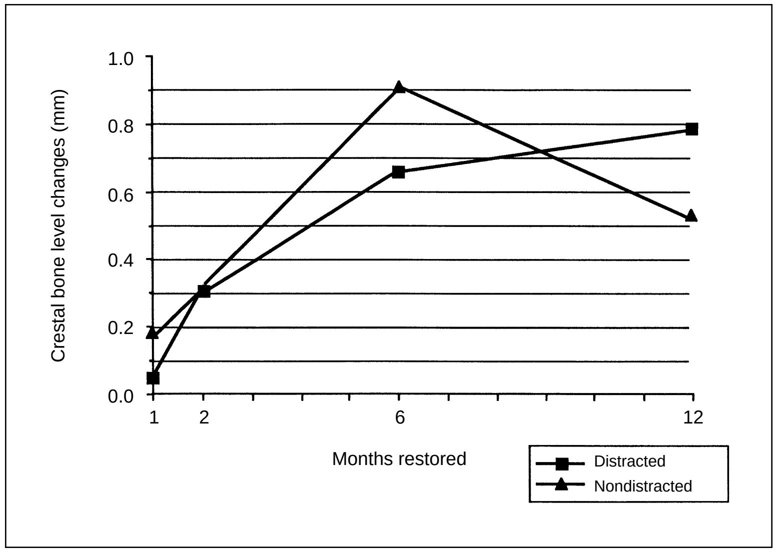


Fig 7 Crestal bone changes over time for implants placed into distracted and nondistracted ridges.

The follow-up examinations were performed under ketamine anesthesia, which is safer for the animals than intravenous pentobarbital. However, one disadvantage of using ketamine as the general anesthetic is small movement of the jaws. This dyskinetic movement resulted in unfortunate movement of the radiograph film, and, as a result of this movement, there was some blurring on the film. Blurred radiographs were not evaluated, hence the difference in N values in Table 1. Because there were some missing data as a result of these blurred radiographs secondary to jaw movement for some of the nondistracted sites, the repeated measures ANOVA to determine significant differences for the treatments over time could not be performed. The change values from baseline for the distracted and nondistracted treatments are presented in Fig 7.

Histologic Evaluation. All four of the dogs appeared to be similar. The cortices were intact and continuous across the distraction gap (Figs 8 and 9). This bone was dense, with lamellar qualities and minimal woven bone. The bone along the labial and lingual cortices was intact between the distraction segments in all four animals. The tissue in the marrow spaces was predominantly fatty marrow. There were no qualitative differences in the cancellous bone regions between the distracted and nondistracted tissues. Implants that were placed close to the cortices had dense bone in the threads, whereas those placed in the middle of the marrow space, without direct contact with cortical bone, had less bone within the threads. This observation was independent of whether the tissue block was from distracted or nondistracted bone.

The bone that spanned the distraction gap superiorly to inferiorly had nutrient canals parallel to the vertical distraction movement. This was clearly evident in all of the sections. There was no evidence of resorption of the superior piece of distracted alveolar bone. Multiple osteoblasts lined the endosteal surfaces of these sections, indicating an active process of bone deposition (Fig 9).

Thickness of Cortical Bone. The thickness of the labial and lingual cortical bone was measured using the Olympus Cue-2 image analysis system. The measured tissue sections included the section through the middle of the implants and a representative section chosen from the tissue blocks that did not have implants. For each section, three measurements were recorded of the cortical bone thickness for the lingual and labial cortices. The three measures of the labial and lingual cortices were averaged and then analyzed (Table 2).

The means for each cortex for the four groups (implant in distracted bone, no implant in distracted bone, implant in nondistracted bone, and no implant in nondistracted bone) were compared using the linear model system of SAS (Statistical Analysis System, Cary, NC). Comparisons were made between distracted and nondistracted bone, implant and no implant, labial and lingual bone, and between dogs.

For all measures together, the general linear models procedure indicated that there was a significant difference between distracted and nondistracted bone (*df* 1, F = 15.35, *P* = .0009), and between labial and lingual bone (*df* 1, F = 23.11, *P* = .0001). There was also a significant interaction between distraction and lingual and labial bone loca-

Figs 8a to 8d Photomicrographs of cross sections of mandibles, with and without implants.

Fig 8a *(Left)* Cross section of mandible, distracted ridge, with implant in place (1:1; undecalcified; Alizarin red).

Fig 8b *(Right)* Cross section of mandible, distracted ridge, no implant in place (1:1; undecalcified; Alizarin red).

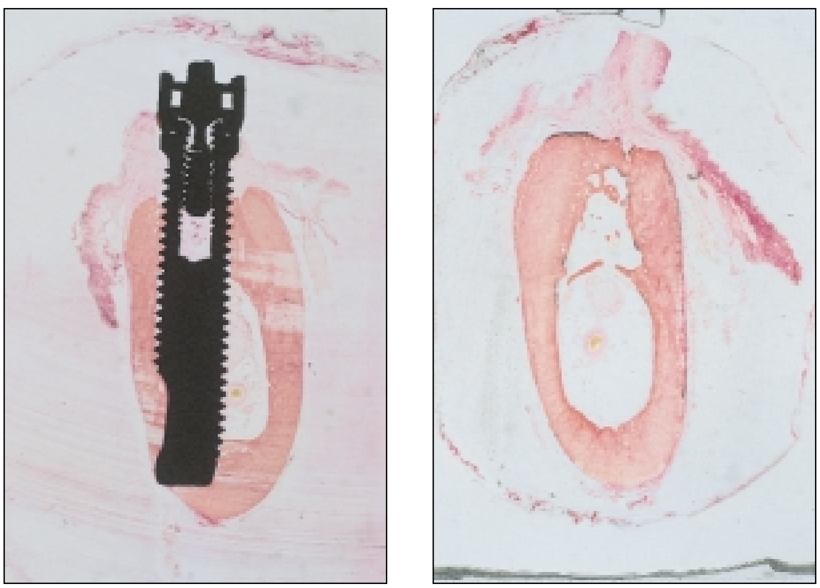


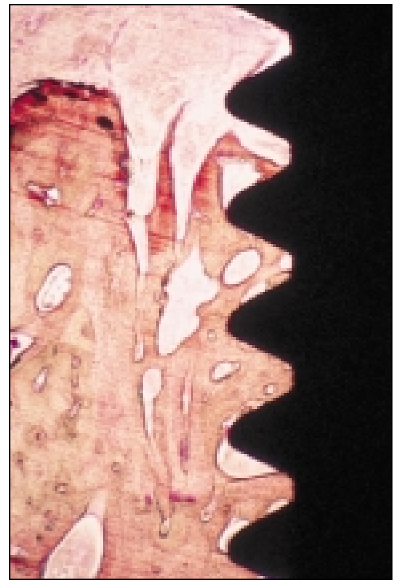
Fig 8c *(Left)* Cross section of mandible, nondistracted ridge, with implant in place (1:1; undecalcified; Alizarin red).

Fig 8d *(Right)* Cross section of mandible, nondistracted ridge, no implant in place (1:1; undecalcified; Alizarin red).

tion (*df* 1, F = 14.55, $P = .0012$). There were no significant differences between implant and no implant, between dogs, or in the interaction between distraction and implant or between implant and lingual or labial bone location.

Duncan's multiple range test was used to compare the four conditions listed above. There was a significant difference in cortical thickness (*df* = 19, $\alpha = .05$, mean square error = 65738.81, $P < .05$) of distracted bone (mean = 1303.8) and nondistracted bone (mean = 1671.5). There was a significant difference (*df* = 19, $\alpha = .05$, MSE = 65738, $P < .05$) in the thickness of the lingual cortex (mean = 1700.47) and the labial cortex (1250.4). There was no significant difference in cortical thickness comparing the presence of an implant to no implant, or between the four dogs.

The general linear models system was then used to compare the labial cortex thickness: for distracted and nondistracted bone; for implant versus no implant; and between dogs. There was a significant difference for the labial cortex bone thickness between distracted (mean = 1637.3 μm) and nondistracted



Figs 9a to 9d Photomicrographs showing newly formed bone around and within the threads of the implants.

Fig 9a *(Left)* Photomicrograph of interface between the inferior intact mandible and the bone formed in the distraction gap (magnification × 25; hematoxylin and eosin; decalcified). Note the vertical orientation of the nutrient canals within the newly formed bone.

Fig 9b *(Right)* Photomicrograph showing the bone within the threads of the implant at the superior aspect of the distracted ridge (× 25; hematoxylin and eosin; decalcified).

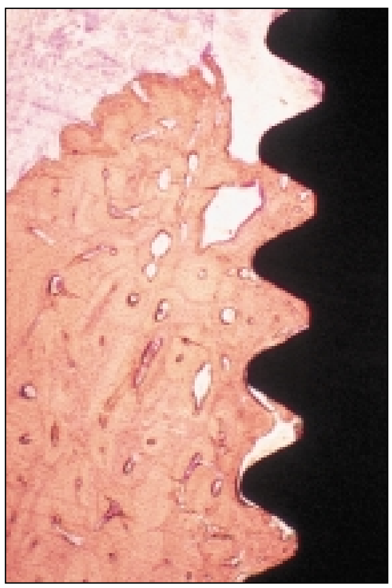


Fig 9c *(Left)* Photomicrograph showing the bone within the threads of the implant at the superior aspect of the nondistracted ridge (× 25; hematoxylin and eosin; decalcified).

Fig 9d *(Right)* Photomicrograph of bone within the threads of the implant, in the newly formed bone within the distraction gap (× 25; hematoxylin and eosin; decalcified).

Table 2 Cortical Bone Thickness of Distracted and Nondistracted Bone, With and Without Implants

Variables	Mean (µm)	SD (µm)
Distraction; no implant; labial	921*	263
Distraction; no implant; lingual	1685	323
Distraction; implant; labial	1023*	277
Distraction; implant; lingual	1550	102
No distraction; no implant; labial	1706	76
No distraction; no implant; lingual	1786	208
No distraction; implant; labial	1546	435
No distraction; implant; lingual	1599	175

*Means are significantly different as compared to all other variables by Duncan's multiple range test ($P < .05$).

bone (mean = 911.8 µm) (df 1, F = 22.24, P = .0015). This was confirmed by Duncan's multiple range test (α = .05, df 8, MSE = 88341, $P < .05$). There was no significant difference for labial bone thickness in the presence of an implant (mean no implant = 1313, mean implant = 1178) or between dogs.

The general linear models system was used to compare the lingual cortex thickness: for distracted and nondistracted bone; for implant versus no implant; and between dogs. There was no significant difference between the lingual cortical thickness of bone for distracted and nondistracted bone, for the presence of an implant, or between dogs. This was confirmed by Duncan's multiple range test ($P > .05$).

Discussion

Slow bone movement resulting in bone production without the need for bone grafts has been accomplished in the long bones as well as in the bones of the jaws. Previous work by the present authors and by others in the mandible and maxilla indicate that these bones can be expanded in length and width using distraction osteogenesis principles.[1–24] An earlier study[1] and the current investigation confirm that, in the dog, the alveolar ridge can be distracted superiorly, can heal with cortical bone formation in the distraction gap, and can subsequently be loaded with implant-supported prostheses.

Histologic observations indicated that the labial cortex was thinner in the distracted regions compared to the nondistracted regions. A number of factors may have contributed to the thinner labial cortex found in this study: the repeated surgical procedures with the labial cortex exposed (anchoring implant placement, exposure for impressions, osteotomy, implant placement, exposure for impressions); the location of the implants; or the lack of teeth and normal labial cortex resorption. In spite of a thinner labial cortex, labial implant dehiscence was not present.

The presence of the implant did not result in thicker or thinner cortical bone. Bone preservation of the crestal region was not different between distracted and nondistracted regions. Therefore, it was concluded that the physiologic reaction of the newly formed bone may be similar to that of nondistracted, edentulous bone of the dog.

The sample size in this study was small, and was compromised by small movement and subsequently blurring of radiographs of the animals' jaws during radiographic examination, when they were under ketamine general anesthesia. It is unclear as to why the distortion was present for only a few of the nondistracted sites. However, the histologic evaluation was complete and verified the hypotheses that the bone remodeled, and that the only significant difference between distracted and nondistracted bone was the thickness of the labial bone.

How much bone height augmentation was preserved over time and what happened to the crestal bone as it was distracted superiorly? The osteotomy was created with the vertical cuts approximately perpendicular to the alveolar crest. To preserve the blood supply to the crest, periosteal reflection was minimal and no bone markers were placed on the top of the crest. After 10 mm of distraction, there existed a step from the nondistracted ridge to the superiorly distracted segment (Fig 6b). Bone fill occurred over the subsequent 6 to 8 weeks. All radiographs, when superimposed, indicated stability of the implants used for anchorage. The distance between them did not change significantly. The implants for support of the prostheses were then placed, slightly countersunk in relation to the crest. During the time for bone fill in the distraction gap, as well as the time between implant placement and integration, the step remodeled to a smooth transition between the nondistracted and distracted alveolus, as is visible in the radiographs. During the loading phase, bone resorbed to the first or second thread of the implant in both distracted and nondistracted bone, as is shown in the radiographs. The adjacent bone was higher than the bone adjacent to the implant as expected, since bone is known to resorb to the first retentive thread of a threaded implant. If the implants had not been countersunk, it is possible that the crestal levels would have been higher.

The bone over the implants that were used to anchor the distraction device can be used as a measure of the preservation of the crestal augmentation, but since they were adjacent to the step of the bone segment, and since this area remodeled into a smooth transition to the nondistracted adjacent bone, it is not known how much of the bone height was affected by the neighboring remodeling of the distracted segment of alveolar crest.

Creation of bone in a transverse axis to its corpus length can be accomplished. Potential applications for moving bone in different vectors include the reconstruction of horizontal and vertical defects of alveolar ridges in partially and completely edentulous subjects. The engineering of different anchoring systems with practicality for clinical use should be paralleled with carefully designed prospective studies.

Acknowledgment

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