Distraction Osteogenesis Versus Autogenous Onlay Grafting. Part II: Biology of Regenerate and Onlay Bone

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Purpose: Few studies have directly compared the quality of bone generated by distraction osteogenesis with that generated by autogenous onlay grafting. The purpose of this study was to compare rates of bone turnover at 5 months in bone produced by distraction osteogenesis and onlay grafting. Materials and Methods: Alveolar defects created in jaws of American foxhounds were augmented with distraction osteogenesis or onlay grafting and allowed to heal for 5 months. The animals were then sacrificed and the jaws were resected and prepared for decalcified and undecalcified histologic examination. Results: Both procedures produced bone containing a mixture of haversian systems and trabecular bone. A significantly greater ratio of osteoblast-covered bone surface to total trabecular bone surface (mean ± SEM) was noted in distraction bone (0.124 ± 0.049) compared to onlay bone (0.081 ± 0.048) or control host bone $(0.085 \pm 0.042 \ \mu m)$ (P < .05). In addition, significantly (P < .05) greater numbers of osteoclasts per μ m of bone surface were noted in distraction bone (0.939 ± 0.07) compared to onlay bone (0.605 ± 0.06) or control host bone (0.725 ± 0.08) . No differences in rates of mineralization were noted between the groups. **Discussion:** While bone from both experimental groups appeared adequate for implant placement, distraction bone appeared to be remodeling at a higher rate than either onlay or control bone. Conclusion: Given that the state of healing of the bone in each of these comparative groups was examined at a static point in time, it is premature to draw conclusions about the efficacy of one procedure over the other. (Basic Science) INT J ORAL MAXILLOFAC IMPLANTS 2006;21:237-244

Key words: alveolar bone, distraction osteogenesis, onlay bone grafting

Autogenous bone grafts are currently considered the "gold standard" for vertical ridge augmentation. The common intraoral donor sites include the maxillary tuberosity and the mandibular ramus or chin. If intraoral bone sites are insufficient, autogenous bone may need to be taken from sites distant from the oral cavity such as the iliac crest, tibia, or rib.^{1,2} Autogenous harvesting techniques have limitations and drawbacks, such as pain and risk of altered function at the donor site or paresthesia/ dysesthesia associated with nerve manipulation.³ The recipient site must be prepared to receive the graft without placing undue stress on the soft tissue closure.⁴ A frequent limitation of vertical onlay block grafting is the unpredictability of increasing the vertical bone height more than 3 to 4 mm.⁵

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A relatively new distraction osteogenesis technique for vertical bone augmentation in the maxilla and mandible may provide several advantages over conventional bone grafting techniques. Distraction osteogenesis is a procedure in which new bone formation occurs between surfaces of bone that are gradually separated by incremental traction that generates tension to stimulate new bone formation parallel to the vector of distraction.⁶ One benefit of alveolar distraction is augmentation of the implant site without the need for a secondary donor site.

Following surgical osteotomy, initial resorptive changes occur at the osteotomy margins because of the initial compromised vascularity. However, the process of distraction osteogenesis may be considered primarily anabolic. This is because the process of distraction results in osteoblast proliferation and enhanced bone formation rather than osteoclastic bone destruction, as initially seen in onlay grafting.⁷ Distraction osteogenesis utilizes a pedicle grafting technique in which the hard tissue segment is never completely severed from its original vascular supply. This pedicle-type graft minimizes osteoclastic resorption of the graft, confining osteoclastic resorptive activity to removal of necrotic tissue at the edges of the bone margins of the osteotomy site. It facilitates maintenance of vitality and maximizes regenerative outcome.⁸ The reparative callus formed early in the distraction osteogenesis procedure contains the necessary osteogenic precursor cells and growth factors to promote bone growth.⁸ Growth factors, such as transforming growth factor (TGF)-β1, produced during distraction osteogenesis promote collagen production, decrease the degradation of extracellular matrix molecules, stimulate osteoprogenitor cells, and inhibit osteoclastic activity at the distraction site.^{8,9} Increased concentrations of bone morphogenetic protein-2 (BMP-2) are seen in distraction osteogenesis,¹⁰ while serum levels of insulin-like growth factor (IGF)-1 are increased early in the distraction period, and further increases in the skeletal levels of IGF-1 are found in the distracted callus and surrounding bone. When stimulated by the controlled tension of distraction, these components generate bone that subsequently undergoes remodeling.8

The early phase of the autogenous block graft incorporation is primarily catabolic (ie, nonpedicle/ free graft), with initial osteoclastic resorption of the grafted bone.^{11,12} The initial phase of healing for free bone grafts includes resorption of the outer surface of the graft, which results from lack of a direct blood supply.^{11,12} Cellular necrosis and osteoclastic activity are increased during the initial stages of wound healing of free block grafts.^{11,12} Once functioning vasculature is re-established within the graft, bone regeneration occurs, followed by remodeling. The cellular activity of the bone and timing of the anabolic and catabolic events that occur during distraction osteogenesis or bone grafting are critical in determining the timing of dental implant placement and the integrity of implant integration.

Snyder and associates¹³ were the first to successfully utilize the canine model to study the principles of distraction osteogenesis in the intramembranous craniofacial bone. Since then, there have been numerous studies using the canine model to examine distraction osteogenesis. The goal of this study was to compare the levels of anabolic and catabolic cellular activities in mandibular bone augmented by distraction osteogenesis versus mandibular bone augmented by iliac crest autogenous onlay graft in a foxhound model. This was accomplished by quantifying the differences in number of osteoclast and amount of osteoblast-covered bone surface relative to total bone surface.

MATERIALS AND METHODS

Animal Model and Surgical Procedures

Five adult male American foxhound dogs weighing between 25 and 30 kg were used in accordance with the guidelines of the Institutional Animal Care and Use Committee at Baylor College of Dentistry. Animals were cared for and surgery was conducted as described in part I.¹⁴ Bilateral extraction of the mandibular premolars and second and third molars was performed after the induction of anesthesia using ketamine HCI (20mg/kg intramuscularly [IM]; Fort Dodge Animal Health, Overland Park, KS) and xylazine (2mg/kg IM; Ben Venue Laboratories, Bedford, OH). After intubation, general anesthesia was maintained with a mixture of 2% halothane (Butler, Dallas, TX) and oxygen at a rate of 1 L/min. The general anesthetic was delivered and monitored under the supervision of an experienced animal technician. The wounds were allowed to heal for 8 weeks. Thereafter, ridge augmentation by distraction osteogenesis was performed on the left side. At the beginning of the consolidation period, 17 days after placement of the distraction devices, ridge augmentation by autogenous onlay grafting into created defects was accomplished on the right side. Onlay grafts from the ilium of each dog were harvested, measured, and shaped to give 10 mm of height. The width was standardized to the maximum width that could be created for the distraction procedures. Using dogs of the same age and weight minimized discrepancies in ridge height between dogs. The distraction device was designed to ensure that 0.5

mm turns twice daily provided the desired final height of 10 mm. After 12 weeks of healing, 4 endosseous implants were placed in each augmented alveolar ridge, and 8 weeks later, the dogs were sacrificed and the jaws resected. Each animal was given 25 mg/kg tetracycline HCl intravenously (IV) 14 days prior to sacrifice and 10 mg/kg IV calcein (Sigma Chemical, St Louis, MO) 3 days prior to sacrifice as vital bone labels.

Specimen Collection

Sacrifice of the experimental animals was performed 20 weeks after autogenous onlay graft surgery and initiation of the consolidation period for the distraction osteogenesis group. At the time of specimen collection, animals were anesthetized with ketamine HCl 20 mg/kg and 2 mg/kg xylazine IM. Once the animals had been anesthetized, they were sacrificed using a mixture of 390 mg/mL phenobarbital sodium and 50 mg/mL phenytoin sodium (Beuthenasia-D, Schering-Plough, Kenilworth, NJ) at a dose of 1 mL/5 kg. The dogs were perfused with 4% paraformaldehyde through the carotid arteries. The mandible was removed en bloc using a bone saw (Stryker, Kalamazoo, MI), and blocks containing alveolar bone from each of the 3 study groups (distraction osteogenesis, onlay graft, control) were prepared in duplicate for decalcified and undecalcified tissue preparation and stored in numbered vials containing perfusion solution. Blocks containing the implants were prepared separately for analysis of bone-implant contact and bone turnover.

Preparation of Undecalcified Bone and Measurement of Rate of Mineralization

In order to determine rates of mineralization, 1 set of bone specimens was embedded in methylmethacrylate resin for undecalcified sectioning and stained with Stevenel's blue. Sections were evaluated using a Nikon Labophot Brightfield microscope (Nikon, Tokyo, Japan) with a Nikon Super High Pressure Mercury Lamp (model LH-M100CB-1) for epifluorescence. The first image was captured using a 405-nm filter to capture tetracycline uptake. The second image was captured using a 490-nm filter to detect calcein uptake. Each set of 2 images was then superimposed and color encoded into a single image, with tetracycline as green and calcein as yellow/orange. The images were then transferred to Adobe Photoshop (Adobe, San Jose, CA) and printed for evaluation using a digital Boley gauge that was accurate to the nearest tenth of a millimeter. For each image the distance of the fluorescent labeling from the edge of the bone to the rear edge of the fluorescent label was measured by an investigator blinded to specimen identity, specimen location, implant number, and group number. This measurement was performed on 3 to 5 randomly selected areas for each image in the upper, middle, and lower regions of the augmented bone, and the measurements were logged in a spreadsheet for analysis. The mean value was obtained and divided by the number of days from the initial staining to the day of sacrifice to calculate the rate.

Decalcified Bone Preparation

To fix the specimens to be analyzed using hematoxylin-eosin (H&E), tartrate-resistant acid phosphatase (TRAP), and modified Attwood's staining, they were placed in 10% formalin to fix the specimens for 24 hours.

Specimens were then transferred to a 0.5 mol/L EDTA solution for decalcification at 4°C. The EDTA solution was changed every 14 days to ensure adequate decalcification of the block sections. Radiographs were taken once a month to evaluate the extent of decalcification prior to histologic processing.

Once decalcification had been confirmed, the specimens were processed for paraffin embedding, and 6- μ m sections were cut through each embedded specimen. Every 6th section of each specimen was stained with H&E for initial histological evaluation. It was then determined which sections would be further analyzed after TRAP and Attwood's staining procedures. The sections were chosen based on the presence of anatomical markers, such as the inferior alveolar nerve; the presence of mucosal tissue on the inferior and superior portion of the sections; and the presence of intact cortical bone on the inferior and superior portions of the section.

TRAP Staining

Selected slides were stained following the TRAP protocol described by Minkin¹⁵ and Burstone.¹⁶ The sections were deparaffinized with xylene and rehydrated with decreasing concentrations of ethanol. Phosphate-buffered saline was used to rinse the sections prior to application of the TRAP stain (naphthol AS-BI phosphate in N,N-dimethylformamide, 0.2 mol/L sodium acetate, fast red violet LB, 10% MgCl₂). Prior to adding to sections, the solution was heated to 37°C and 50 mmol/L L-(+)-tartaric acid was added. The TRAP solution was applied to the slides and incubated at 37°C for 2 hours in a moist chamber. Sections were rinsed with water and counterstained with hematoxylin and light green stain. The slides were then dehydrated in ethanol, cleared in xylene, and covered with coverslips placed with Permount (Sigma).

Modified Attwood's Stain

Analysis of tissue sections adjacent to TRAP sections was performed with the modified Attwood's phloxine-tartrazine stain for analysis of the demineralized paraffin-embedded bone as described by Putns and Desa¹⁷ and Hess and Villaneuva.¹⁸ The Attwood's stain made the phloxine-positive lamellar bone easily distinguishable from the tartrazine-positive woven bone.

Analysis of Specimens and Data Collection

Digital imaging techniques were used to visualize the spatial arrangement of the newly formed tissues. The TRAP-stained sections and modified Attwood'sstained sections were digitized with a Spot camera (Eastman Kodak, Rochester, NY) mounted on a Zeiss Axiophot microscope (Oberkochen, Germany) for computer-assisted analysis. After importing the images, linear measurements of the bone surfaces were recorded using the integrated morphometry tool in the Metamorph software (Universal Imaging, West Chester, PA).

In the TRAP-stained sections, the TRAP-positive cells (osteoclasts) were manually counted along all measured bone surfaces. The linear surfaces of bone in the Attwood's-stained sections were scored in a similar manner using the Metamorph software. Analysis of the Atwood's-stained sections included measuring the surface of bone covered by osteoblasts and comparing these data to total bone surface in the sections using the Metamorph Imaging System.

Data sets were logged into Excel spreadsheets (Microsoft, Redmond, WA) for statistical analysis and are expressed as means \pm standard errors of the mean (SEM). Statistical analysis was performed to compare ratios of osteoblast-covered surface to total bone surface, using analysis of variance (ANOVA) for the initial detection of significant differences among the 3 groups, followed by the Fisher's protected least significant differences. The Fisher's PLSD allows for comparison of multiple groups 2 groups at a time. The null hypothesis was rejected whenever the statistical probability rated below 5% (P < .05).

RESULTS

Bilateral vertical ridge augmentation was successfully performed utilizing autogenous onlay grafts harvested from the iliac crest and the distraction osteogenesis procedure. Clinical evidence of vertical distraction osteogenesis was noted prior to the implant surgery. The design of the distraction device ensured that 10 mm of ridge height was consistently achieved in all dogs. On visual inspection, no evidence of loss of bone height was noted in any of the onlay grafts. No gross measurements of bone height or volume were made, as visual inspection did not reveal differences between specimens. Intraoral inspection of the soft tissues adjacent to the distraction screws revealed erythematous and edematous tissues (See Fig 2 of part I of this article¹⁴).

Histological Analysis of Bone

The control group revealed a mature architecture, with well-developed haversian systems, as well as partially resorbed haversian systems (not shown). A moderate amount of trabecular bone was noted within the control sections. The distraction osteogenesis group had a similar histologic appearance to that of the control sections, with the presence of mature and partially resorbed haversian systems and some trabecular bone (Figs 1a and 1b). One difference in the overall anatomy of the distraction osteogenesis sites was the presence of buccal concavities in areas of the osseous regenerate (not shown).

The autogenous onlay graft sections revealed a somewhat different histologic morphology. Several mature haversian systems were noted, and distinct reversal lines were visible at the boundary between onlay bone and newly formed bone (Figs 1c and 1d). Many empty lacunae were also noted within the onlay bone (Fig 1d).

Analysis of Amount of Osteoclasts

Osteoclasts were detected throughout the bone in all 3 groups and were especially prevalent in areas where cancellous bone predominated (Fig 2). The osteoclast/trabecular bone surface ratio (mean + 1 standard deviation) was 0.725 + 0.08 in control bone versus 0.939 + 0.07 in distracted bone; this was a significant difference (P < .05) (Fig 3). The osteoclast/trabecular bone surface ratio in onlay graft bone was 0.605 ± 0.06 ; this ratio was not significantly different from control bone. However, the mean osteoclast/ trabecular bone surface ratio in onlay graft bone was significantly lower than that seen in distraction bone (P < .05).

Analysis of Osteoblast-Lined Bone Surface Versus Total Bone Surface

Osteoblasts were seen lining trabecular bone surfaces, and the presence of osteoid was detectable underneath the osteoblast layer (Fig 2b). The mean ratio of osteoblast-lined bone surface to total trabecular bone surface in the control group was 0.085 + 0.042 versus 0.124 + 0.049 in the distraction osteogenesis group; this was a significant difference (P < .05) (Fig 4). The mean ratio of osteoblast-lined surface to

Figs 1a to 1d Photomicrographs of H&E-stained histologic sections through bone created by distraction osteogenesis and onlay graft bone. (a) Low-power (\times 5) image of distracted bone, showing the presence of many haversian canals. Osteoblasts can be seen lining free bone surfaces. (b) Low power (\times 5) image of onlay bone showing newly formed woven bone adjacent to the onlay block graft (arrows). (c) Higher-power (\times 10) image of bone section shown in a. (d) Higher power (\times 10) image of bone section shown in b, showing the presence of empty lacunae in the block graft (arrowheads).



Fig 2 (a) Photomicrograph of a section through distracted bone regenerate stained with TRAP. Note the presence of many multinucleated osteoclasts (arrows). (b) Photomicrograph of an onlay graft bone section stained with Attwood's stain. Note the presence of osteoid (pale yellow) underneath the osteoblasts lining the bone surfaces (arrowheads). (c and d) Photomicrographs of sections of undecalcified distraction and onlay graft bone, respectively, showing tetracycline (red) and calcein (green) labeling. Note the white bars denoting examples of positions at which measurements were taken (asterisks). Note the close association between the bone and implant (arrows). (Original magnification of all sections \times 20).





Fig 3 Graph showing the number of osteoclasts per μ m bone in the control, distraction osteogenesis, and onlay grafting groups. Error bars = SEM. **P* < .05.

total bone surface in the onlay graft group was 0.081 + 0.048; this was not significantly different from the control group, but it was significantly different from the distraction osteogenesis group (P < .05).

Rates of Mineralization

Tetracycline and calcein labeling was clearly detectable in all groups, showing a high rate of mineralization for all groups (Figs 2c and 2d). Rates of mineralization were measured in the upper, middle, and lower thirds of the augmented bone as described in the Methods section. Mean rates of mineralization varied from 1.64 ± 0.14 to $2.21 \pm 0.16 \ \mu\text{m}$ per day in the lower third, from $1.70 \pm$ $0.15 \text{ to } 2.07 \pm 0.15 \ \mu\text{m}$ in the middle third, and from $1.67 \pm$ $0.13 \text{ to } 2.0 \pm 0.0 \ \mu\text{m}$ in the upper third of all groups (Table 1). No statistical differences were found for the rates of mineralization either between groups or between regions (*P* > .05).

DISCUSSION

The placement of dental implants requires sufficient quality and quantity of bone along with sufficient soft tissue volume to mimic gingiva adjacent to natural teeth. Distraction osteogenesis is a technique whereby bone is augmented while soft tissue is expanded to a level that existed prior to tooth loss and subsequent residual ridge resorption. Distraction osteogenesis allows for the vertical augmentation of the alveolar ridge without a secondary donor site and can be performed on an outpatient basis. In contrast, current onlay bone grafting techniques require bone harvest from secondary sites. Those sites could be local (ie, the chin or mandibular ramus) or distant from the oral cavity. The addition of surgical sites for a patient results in increased potential for surgical risks.^{1,2}



Fig 4 Graph showing μ m of osteoblast-covered surface per μ m total bone surface in the control, distraction osteogenesis, and onlay grafting groups. Error bars = SEM. **P* < .05.

Frequently, patients requiring dental rehabilitation have complex ridge deformities in the vertical and/or horizontal dimensions that require ridge reconstruction prior to dental implant therapy. During treatment planning, the surgeon must evaluate whether onlay block grafting or distraction osteogenesis would be the preferred treatment modality based on the specific clinical situation. These 2 ridge augmentation therapies differ in several ways. The block graft harvested from an extraoral donor source is a free bone grafting technique, while the distraction osteogenesis procedure utilizes a pedicle grafting technique in which the hard tissue segment is never completely severed from its original vascular supply. This pedicle-type graft is designed to minimize resorption of the graft, facilitate maintenance of vitality, and maximize the regenerative outcome.¹⁹ During the distraction process, tensional stresses are transferred to the contiguous soft tissues, triggering new soft tissue growth and adaptation.¹⁹ Thus, the treatment option of distraction osteogenesis carries with it several advantages for the clinician as well as for the patient.

The current study showed that either onlay grafting or distraction osteogenesis could be used to augment alveolar ridges. However, bone from both procedures showed some degree of trabeculation, and in the distraction osteogenesis group, some buccal concavities were noted. These buccal defects had no effect on the amount of implant integration, which was described in part I.¹⁴ No measurements of bone volume were made, because no differences in degree of osseointegration of implants were found when implants in the distraction groups were compared to implants in the onlay bone groups, and implants in both groups appeared to have better osseointegration than implants in the control group.¹⁴ In studies performed by Block and colleagues,^{20,21} results indicated that the distraction gaps did not heal with significant bone fill at the 6-week time point. These authors also noted the appearance of buccal concavities with distraction osteogenesis. While a longer period of time was used in the present study, it is likely that bone healing was not complete 3 months after implant placement.

The 6-week length of time used by Block and associates^{20,21} coincided with preliminary studies which investigated implant integration into distraction regenerate, which indicated that 6 weeks was an adequate period of time for osseointegration to occur. However, Buser and coworkers²² found that degree of implant integration appeared to depend on the implant surface used (machined versus rough). Data reported in part I of this study showed that bone-implant contact was high, supporting the notion that the residual presence of trabeculation need not compromise implant stability.

The presence of significantly higher numbers of osteoblasts and osteoclasts in the distraction osteogenesis group showed that greater bone turnover was occurring in this group than in the control or onlay grafting group. This finding was similar to that of Tay and colleagues,²³ who found active bone remodeling within the regenerate tissues. In contrast, Cope and Samchukov²⁴ described complete bone formation within the distraction gap at 8 weeks of consolidation. However, that study²⁴ examined bone regenerate in horizontally distracted bone, and the differences in bone formation may be due to varying stresses placed on the bone in these 2 procedures.

In distraction osteogenesis, the bone within the distraction gap is primarily newly generated bone that forms intramembranously.²⁵ The trabeculae form essentially as open-ended osteons, which allow newly forming blood vessels to grow into the edges of the gap. Newly formed trabeculae begin to thicken at the edges closest to the osteotomy margins, and rapid remodeling of the bones begins to occur at the end of the distraction period and continues through the consolidation period.²⁵

In contrast, onlay bone is essentially severed from its vascular blood supply, resulting in initial cellular necrosis.^{11,12} As a functioning vasculature becomes re-established, bone remodeling of the onlay graft occurs via bone remodeling units. The invasion of osteoclasts into the grafted bone is rapidly followed by an ingrowing vasculature and osteoblastic activity, creating new bone.²⁶ The bone is then remodeled, resulting in the creeping substitution of the bone graft, until the bone graft has been completely replaced by new bone.^{11,12} The higher numbers of osteoblasts and osteoclasts in regenerate bone reported here suggest that the amount of bone

| Table 1 Rates of Mineralization ($\mu m/d$) | | |
|-----------------------------------------------|--------------------------------------------|-------------------------------------------|
| Procedure | Region of regenerate | Mean ± SEM (10 ⁻²) |
| Distraction osteogenesis | Lower Third Middle Third Upper Third | 2.21 ± 0.16 2.07 ± 0.15 1.67 ± 0.13 |
| Onlay graft | Lower Third Middle Third Upper Third | 1.64 ± 0.14 2.00 ± 0.15 1.93 ± 0.12 |
| Control | Lower Third Middle Third Upper Third | 1.90 ± 0.32 1.70 ± 0.15 2.00 ± 0.00 |

Lower third, middle third, and upper third indicate the position within the distraction regenerate or onlay graft where measurements were made, with the "lower third" being the apical third and the "upper third" being the coronal third.

remodeling occurring in the regenerate is higher than that occurring in the onlay grafted bone at 5 months.

Adaptation to the placement of a dental implant by the host bone depends on both bone modeling (change in size or shape) and bone remodeling (internal turnover or replacement of existing bone) as described by Frost.²⁷ This adaptive capacity allows bone to withstand variations in clinical conditions, particularly masticatory stresses. Successful long-term maintenance of endosseous implants involves a sustained increase of bone remodeling in the local region surrounding the dental implant.²⁸ This group found that bone remodeling appeared to be greatest in the bone adjacent (within 1 mm) to the implant interface and decreased with increasing distance from the implant surface. This appeared to be the steady-state condition, because the zone of elevated remodeling was present in implants in place for up to 5 years. The zone of elevated remodeling appears to be necessary to repair local areas of bone microdamage, particularly occurring in bone surrounding screw-type implants.²⁹ If the interface remodeling rate were lower, the ability to repair fatigue damage might also be diminished. An elevated level of remodeling activity is a universal mechanism necessary for long-term retention of integrated implants and suggests that the normal maintenance of a successful implant requires sustained remodeling activity.²⁸

The results of the present study showed a higher level of remodeling activity in the bone regenerate of the distraction osteogenesis group when compared to the bone in the autogenous onlay graft group and the bone from untreated extraction sites. This suggests that in cases of distraction osteogenesis bone is remodeled at a faster rate than in onlay graft or control bone. This information may aid the clinician in making decisions regarding the time between surgical ridge augmentation with distraction osteogenesis and dental implant placement. Further studies should evaluate the differences in the osteoclastic and osteoblastic cellular activities at different time points in distraction osteogenesis and autogenous onlay grafting, examining both loaded and nonloaded implants.

CONCLUSION

Histologic evaluation of bone in a canine animal model demonstrated significantly greater osteoblast and osteoclast concentrations in bone formed through distraction osteogenesis when compared with control bone or grafted bone. However, given that the state of healing of the bone in each of these comparative groups was examined at a static point in time, it is premature to draw conclusions about the efficacy of one procedure over the other.

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