Consolidation Period in Alveolar Distraction: A Pilot Histomorphometric Study in the Mandible of the Beagle Dog

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Purpose: *Osteogenic alveolar distraction remains in an experimental stage. The present study aimed to compare histologic and histomorphometric results with 2 different consolidation periods (4 and 8 weeks) to determine which period obtained better bone quality after distraction with a prototype alveolar distractor.* ***Materials and Methods:*** *Five beagle dogs were used. Four underwent alveolar distraction in an edentulous segment of the right mandible. After a 7-day latency period, distraction was carried out at a rate of 1 mm/d for 5 days; the consolidation period was 4 weeks in 2 dogs (group 1), and 8 weeks in the other 2 (group 2). The fifth dog was used as control (group 3); it underwent removal of its right premolars but not distraction. Histologic and histomorphometric studies were conducted.* ***Results:*** *One animal from each distraction group was withdrawn from the study because of wound dehiscence that allowed invasion of mucosa into the distraction chamber, which was incompatible with bone regeneration. In the group 1 animal, a predominance of immature woven bone was observed in the distraction chamber, whereas the group 2 animal showed a predominance of immature parallel-fibered bone. The group 1 and 2 animals that remained in the study differed in bone area density in the distraction chamber (36.61% ± 9.79% versus 58.72% ± 8.30%), bone perimeter in the distraction chamber (262.89 ± 10.46 mm versus 201.44 ± 22.64 mm), total height attained (21.31 ± 0.32 mm versus 18.37 ± 0.50 mm), lingual trabecular width (134.00 ± 15.56 versus 229.50 ± 29.24), buccal trabecular width (90.00 ± 4.24 mm versus 154.50 ± 21.64 mm), lingual osteoid area density (4.08% ± 0.46% versus 1.61% ± 0.33%), and buccal osteoid area density (3.75% ± 1.28% versus 2.09% ± 0.79%).* ***Conclusion:*** *Quantitative and qualitative differences in newly formed bone were observed after 4 and 8 weeks of consolidation. These preliminary results serve as a basis for further experimental research with larger samples and for clinical studies.* (Animal Study) (More than 50 references) Int J Oral Maxillofac Implants 2006;21:380–391

Key words: *alveolar distraction, consolidation period, experimental oral surgery*

Distraction osteogenesis is defined as the development of newly formed bone and surrounding soft tissue between 2 bone fragments previously separated by osteotomy in such a way that the gap between them is gradually increased without interrupting the blood supply. This process is based on the so-called Ilizarov effects: *(1)* gradual traction of the tissues creates stress, which activates tissue growth and regeneration (law of tension-stress), and *(2)* the shape and mass of the bone are influenced by the mechanical load and blood supply.[1,2] Block and associates[3] carried out the first alveolar distraction in dogs in 1996, and in the same year, Chin and Toth[4] reported the first application of alveolar distraction to humans, patients with trauma-induced dental losses.

The results of studies of the use of vertical augmentation to increase bone availability in the mandibular or maxillary alveolar ridge have been disparate and controversial compared with horizontal augmentation techniques. Initial clinical outcomes suggested that alveolar distraction yields more rapid, predictable, and permanent outcomes compared with other regeneration methods.[5,6]

The consolidation period is defined as the time between the end of the distraction phase and the

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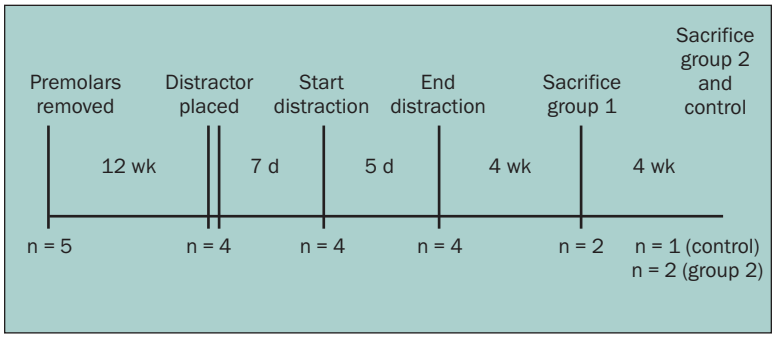


Fig 1 Experiment schedule.

withdrawal of the distractor. This time period allows the immature bone formed in the distraction chamber to become mature bone capable of appropriate function. In the case of the alveolar ridge, adequate biomechanical conditions are sought to enable the placement of dental implants without collapse of the regenerated area. The consolidation period has been studied using different procedures in limb elongation,[7–9] mandibular elongation,[10–17] maxillary elongation,[18–20] and alveolar ridge augmentation.[5,21–23]

Consolidation period length can be based on a number of criteria, including radiologic, histologic, or simply empirical criteria and experimental results. No clear differential criteria have been established in relation to factors with major influence on bone biology, such as gender, age, anatomic localization, bone density, occlusal type, or general health status of the patient. Clinical studies of alveolar distraction have used consolidation times of 4 weeks,[24–27] 8 weeks,[28–32] and 10 to 12 weeks.[6,33–35] Some investigators have proposed the placement of implants immediately after withdrawal of the distractor,[6,26,28–32,34,35] whereas others recommend a consolidation period of 4 weeks.[24,25,27] Very few studies have reported on the appropriate timing of prosthodontic loading, ie, on the appropriate interval between the placement of implants and the start of loading. Intervals of 12 weeks,[29] 16 weeks,[30,36] 24 weeks,[27] and, in the case of the DISSIS distractor-implant (Distraction Implant System, SIS, Klagenfurt, Austria), 16 to 24 weeks have been applied.[37]

Although histologic evaluation is the most objective method to determine appropriate times for distractor withdrawal and implant placement, the use of histologic evidence has been uncommon in published studies. Clinical studies of alveolar distraction have reported immature bone at 8 weeks after distractor withdrawal[24,31] and lamellar bone at either 8 weeks,[29] 10 weeks,[34] or 20 weeks.[28]

The variability of published results makes it difficult to establish the shortest consolidation period that guarantees maintenance of the regenerated bone without collapse or fracture of the area. Most studies have demonstrated that implants can be placed 8 to 10 weeks after the end of distraction.[24,25,28–31,33,34,38] However, some authors have proposed that the presence of a large proportion of immature bone is sufficient to create the correct biomechanical conditions in the distraction chamber for implant placement with adequate primary stability, ie, that it is not necessary to achieve lamellar bone. This has been established in clinical[24,28] and experimental[22] studies of alveolar distraction and in experimental studies of mandibular elongation.[12,13,16] Nevertheless, the appropriate consolidation time to achieve the necessary proportion of immature or type 2 bone remains unknown in both animals and humans.

The present study was conducted to address this issue. The objectives of the study were to assess the viability of a novel prototype for alveolar distraction; to evaluate the clinical and histologic characteristics of newly formed bone in the space created by vertical bone distraction, comparing these characteristics between study groups (4 and 8 weeks of consolidation); and to quantify static histomorphometric parameters in newly formed, basal, and transported bone of both study groups.

The viability of the prototype was assessed by daily clinical examination. The operated areas were also assessed daily to detect regenerated bone and any clinical complications. The histologic and histomorphometric features of the newly formed bone were studied by direct microscopic observation of samples using bone morphometry software (MIP 4 Advanced; Consulting Imagen Digital, Barcelona, Spain).

MATERIALS AND METHODS

Animals

The study used 5 male beagle dogs aged approximately 2 years and weighing between 10 and 15 kg. The study was approved by the Ethical Committee for Animal Experimentation of the Gomez Ulla Central Military Hospital (Madrid, Spain). For the distrac-

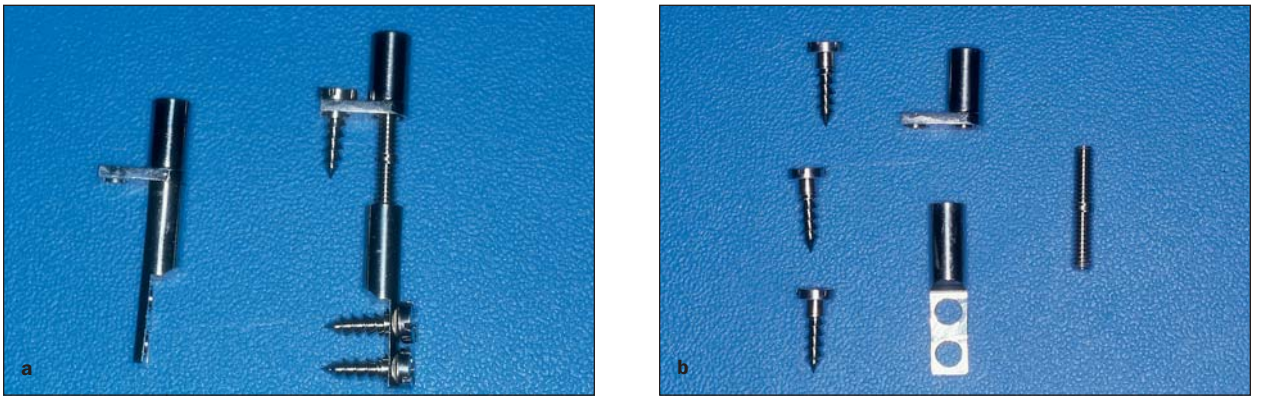


Fig 2 Distractor prototype used *(a)* open and *(b)* dismantled.

tor placement, premedication and induction of anesthesia were accomplished with the intramuscular injection of medetomidine (20 to 40 mg/kg Domtor; Pfizer, New York, NY) and butorphanol (Torbugesic; Fort Dodge, Kansas City, MO) (0.2 to 0.4 mL/kg). Anesthesia was maintained by endotracheal tube administration of 1.5% to 2% isoflurane and a nitrogen dioxide (NO_2) and oxygen (O_2) compound (60% NO_2 and 40% O_2) at a tidal volume of 12 mL/kg.

The experimental animals were randomly assigned to 1 of 3 groups. Two groups contained 2 animals each. A distractor was placed in the right hemimandible of these dogs. The remaining animal (the third "group") served as a control. The experimental study schedule is shown in Fig 1. Group 1 animals were subjected to a 4-week consolidation period after distractor withdrawal, while group 2 animals were subjected to an 8-week consolidation period after distractor withdrawal. The control animal underwent extraction of the right maxillary and mandibular premolars, but no distractor was placed.

In groups 1 and 2, the study parameters were assessed and quantified in the following parts of the distraction area:

- **Area A:** The displaced or transported bone fragment, ie, the upper or occlusal part
- **Area B:** The distraction chamber, ie, the area to be regenerated
- **Area C:** The basal bone fragment

Distraction Equipment

The prototypical distractor used (Fig 2) was designed by the author's research group and manufactured by an implant company (Impladent, Barcelona, Spain). The device, an extra-bone distractor, was made of grade 5 titanium with a machined surface. It comprised a distraction body (12 mm long and 3 mm in diameter) and 2 small anchor plates 0.8 mm thick: a lower one with 2 holes and an upper one with 1 hole. A 6.8-mm fixation screw 1.7 mm in diameter was inserted into each hole. The distractor contained a distraction screw 9.5 mm in length and 1.8 mm in diameter, with each half of the screw threaded in opposite directions. Each 360-degree turn of the distraction screw produced a height gain of 0.66 mm. A maximum distraction of 6.6 mm (10 turns) could be achieved with this distractor before the upper and lower fragments were separated.

Surgical Protocol

All interventions were carried out in an animal operating room under sterile conditions. The 4 right maxillary and mandibular premolars were carefully extracted. Extraction was followed by a 12-week interval for adequate healing of hard and soft tissues. The distractor was then placed in the mandibular alveolar ridge, which was edentulous in the area immediately distal to the right mandibular canine. A full-thickness mucoperiosteal incision was made between the canine and the first molar, which was intracrevicular on the buccal aspect of both teeth. The mental foramen was located, and the buccal mucoperiosteal flap was reflected. An oscillating saw (OMS 5000; Nouvag, Gouldach, Switzerland) was used at a maximum speed of 2,000 rpm with copious saline irrigation to perform a horizontal osteotomy at a distance of 5 mm from the upper alveolar edge. The distraction segment thus created had a mesiodistal length of 20 mm. The osteotomy was deemed complete at the lingual level by palpation of the lingual surface with a fine probe. The distractor was positioned, and holes for fixation screws (mean depth, 6 mm) were drilled under irrigation using a Lindemann tungsten bur 1 mm in diameter (Komet; Gebr Brasseler, Lemgo, Germany). The lower fixation screw was screwed into place, followed by the occlusal screw and, finally, the upper fixation screw of the lower plate. Using a tapered tungsten bur (Komet; Gebr Brasseler) and irrigation, 2 osteotomies were per-

formed of approximately 2 mm depth in the upper area of the alveolar ridge, 20 mm apart and equidistant from the major axis of the distractor, to enable subsequent positioning of the vertical osteotomies and avoid sharp edges on the transported bone fragment. The 2 vertical osteotomies were then performed with oscillating saw and irrigation on the previously marked areas. Cutting of the lingual mucosa was carefully avoided. The distraction device was tested by turning the distraction screw and then returning the distractor to its initial position (Fig 3). The incisions were then closed using modified interrupted mattress sutures with 3-0 nylon (Laboratorio Aragó, Barcelona, Spain). For the next 3 days, all animals received intramuscular injections of a combination of streptomycin and penicillin G (Vetione, Schering-Plough, Segre, France) (2 mL/animal/d) and flunixin (Finadyne, Schering-Plough) (1.1 mg/kg/d).

After a latency period of 1 week, the sutures were removed, and distraction was performed at the rate of 1.5 turns/d (1 mm/d) for 5 days. The distraction was performed under sedation with medetomidine (20 to 40 mg/kg) and butorphanol (0.2 to 0.4 mg/kg); sedation was reversed with atipamezole (Antisedan; Novartis Animal Health, Basel, Switzerland) (20 to 40 mg/kg). On each day of the distraction, the surgical area was evaluated, and any complications were treated. Chlorhexidine in gel and spray forms (Lacer, Barcelona, Spain) was applied once weekly. Biopsy specimens were taken in the operating room of the hospital department prior to sacrifice. A full-thickness supracrestal incision was performed, with removal of the buccal and lingual mucoperiosteal flap in the entire edentulous area. The 3 fixation screws were then unscrewed, and the distractor was removed. A membrane fixation pin (Frios Fixation Set; Friatec, Mannheim, Germany) was placed in the buccal area of the basal bone to position the sample. Using a sagittal saw (ELAN-E; Aesculap, Tuttlingen, Germany) and irrigation with saline serum, a transverse osteotomy of the entire mandibular body was performed, with a distal and mesial margin of approximately 3 mm from the transported bone fragment. Therefore, the bone blocks included the entire distracted bone area and underlying mandibular bone. The animals were sacrificed by intravenous injection of sodium thiopental (1 g/animal) (Abbott Laboratories, Abbott Park, IL).

Clinical, Histologic, and Histomorphometric Evaluations

The state of the mucosa in the distraction area was evaluated throughout the study. The samples were fixed for 7 days in a 4% formaldehyde solution (Panreac Química, Barcelona, Spain) buffered with sodium bicarbonate ($NaHCO_3$) and hydrochloric acid (HCl) at pH 7. The samples were prepared following Donath and Breuner's technique[39] for obtaining bone sections without decalcification.

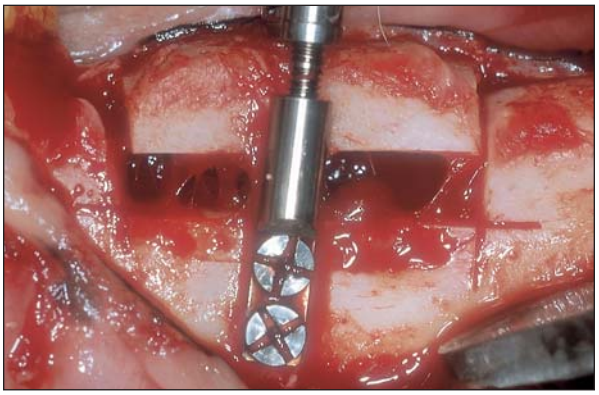


Fig 3 Distraction test (buccal view).

Samples were dehydrated by immersion in solutions containing increasing percentages of ethanol. They were then embedded in glycolmethacrylate resin (Technovit 7200 VLC Embedding Media; Heraeus-Kulzer, Wehrheim, Germany) and kept in an opaque container. After the appropriate time interval for the embedding, the samples were light-cured in a polymerization unit (Light Polymerization Unit; EXAKT Vertriebs, Nordestedt, Germany).

Sections 180 mm thick were obtained by means of a cutting unit (Cutting Grinding System, EXAKT Vertriebs, Nordestedt, Germany) using a saw with a 0.2-mm-thick blade. Sections in each sample were made transversally to the longitudinal axis of the sample (perpendicular to the mandibular body), producing distal sections of the sample. The samples were polished in a polishing unit (Micro Grinding System; EXAKT Vertriebs) to produce samples with a thickness of 30 to 90 mm. They were then stained using the toluidine blue technique and Goldner's modification of the Masson trichrome technique. Donath's special procedure for methacrylate-embedded calcified bone was applied.[39]

The histologic study was performed with an optical microscope (III RS; Carl Zeiss, Oberkochen, Germany) at magnifications of 40×, 100×, 200×, and 400× using filters to polarize the light. The following qualitative variables were examined in the distraction chamber (area B): woven bone, parallel-fibered bone, lamellar bone, trabecular structure, cortical structure, continuity of lingual surface, and continuity of buccal surface. The amount of each of these was estimated to be one of the following: < 25%, 25% to 50%, 50% to 75%, or > 75%.

Various static histomorphometric and bidimensional quantitative variables were also measured in areas A, B, and C, or in area B alone. The nomenclature and abbreviations used follow the recommendations of the American Society for Bone and Mineral Research.[40]

Capture of the images was carried out using a video camera (SICOLOR C810; Siemens, Munich, Germany) connected to a microscope (WILD M7A; Leica, Hildesheim, Germany), storing the images in a computer. The density of bone area, bone perimeter, and bone height were measured with capture at 5× magnification. The buccal and lingual trabecular widths and densities of buccal and lingual osteoids were studied in the distraction chamber at 30× magnification.

A software package for histomorphometric processing (MIP 4 Advanced; Consulting Imagen Digital) was used to obtain the results for all quantitative variables studied. The measurements were obtained by line demarcation and segmentation with gray tones. The following quantitative variables were studied:

- **Bone Area Density:** The bone area density was the amount of mineralized bone in both trabeculae and osteons relative to the total tissue area, expressed in mm^2 or as a percentage. It was determined in areas A, B, and C by dividing section area by total area and multiplying by 100 to obtain a percentage.
- **Bone Perimeter:** The perimeter of the whole area of mineralized trabecular or cortical bone, expressed in mm. It was determined for areas A, B, and C. The final value was the mean of the values obtained in all the sections of each sample.
- **Bone Width:** The bone width or height, expressed in mm, was obtained in areas A, B, and C and for the whole of the sample,. The 3 study areas were demarcated by lines, and 3 equidistant measurements were made in each area, as well as in the whole sample, following an occlusal-basal direction. The mean of the 3 values obtained was gathered for each sample, and the final value was the mean of the values obtained in all the sections of each sample.
- **Buccal and Lingual Trabecular Width:** These variables were calculated for area B. These described the formation and thickness of the trabeculae of the regenerated area, expressed in μm. They were determined in an 1800 × 1800-μm field in the buccal and lingual areas of the chamber adjacent to the basal bone. Five measurements were made in the intermediate width area of 5 equidistant isolated trabeculae, and the arithmetic mean was calculated. The final value was the mean of the values obtained in all sections of each sample.
- **Density of Buccal and Lingual Osteoid Areas:** These variables were calculated for the distraction chamber. This determines the amount of osteoid in both trabeculae and osteons relative to the total area of tissue in the field studied, expressed as a percentage. These values were obtained based on measurements of an 1800 × 1800-μm field in the buccal and lingual areas of the chamber adjacent to the basal bone. They were calculated by dividing the osteoid area by the total area (3,200 μm^2) of the field under study.

RESULTS

All 5 animals recovered well from the surgery, and their feeding and general health status was good throughout the experiment. In 2 animals, 1 each from groups 1 and 2, a dehiscence of the surgical wound with exposure of the transported bone fragment was observed when distraction was scheduled to commence. The area was irrigated with saline and sprayed with chlorhexidine, and the wound was closed with resorbable 3-0 nylon (Vicryl; Johnson & Johnson/Ethicon, Somerville, NJ). In following days, the area again became exposed, and it was decided to withdraw the distractor and transported bone fragment without starting the distraction. The mucosa was again sutured, and the animals were not sacrificed.

The distraction phase was completed in the 2 remaining animals, and vertical augmentation of the alveolar ridge of approximately 5 mm was achieved. At examination, this increase was observed in both the hard tissues and the overlying mucosa (Fig 4). No resistance to the turning of the distraction screw was observed during the first 2 days of the distraction phase, but resistance was felt in the last 3 days.

In the group 1 animal, a small mucosal dehiscence was observed on the mesial aspect of the distracted area during the first week of consolidation. The area was treated once weekly with chlorhexidine gel and spray. The dehiscence did not increase, and the distractor was stable during the 4-week period.

At the biopsy specimen extraction, the distraction chamber contained tissue that was hard (although less hard than the basal bone). A depression was observed on the buccal aspect of the distraction chamber; a more shallow depression was observed on the lingual aspect.

In the group 2 animal, inflammation was observed in the mucosa adjacent to the occlusal fixation screw during the fourth week. This inflammation produced a mucosal dehiscence that allowed communication

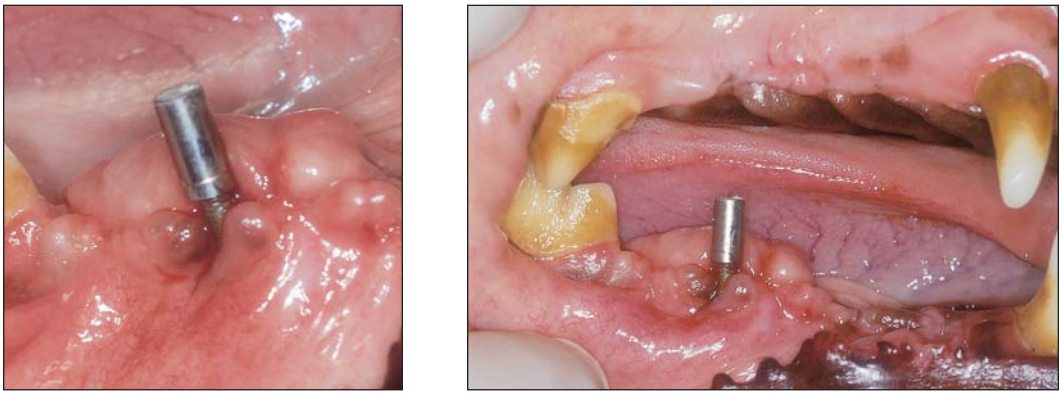
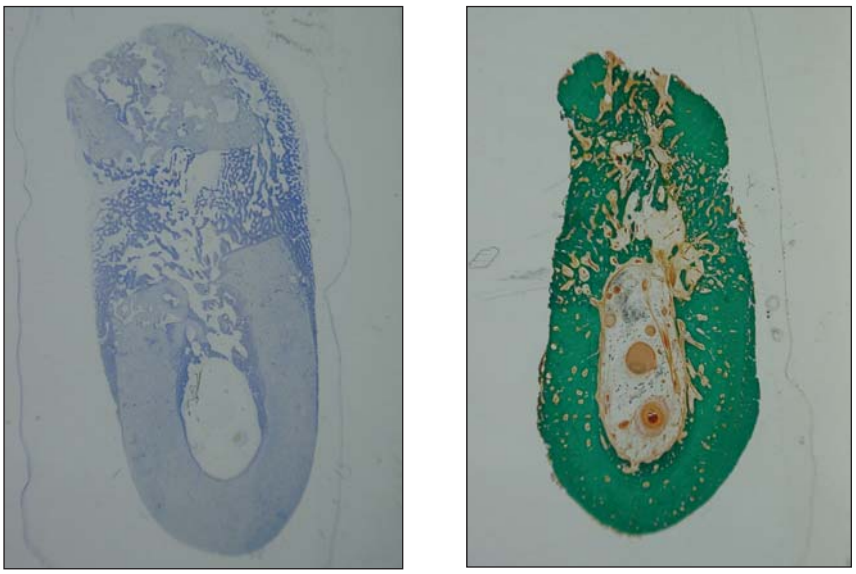


Fig 4 Augmentation of the alveolar ridge at the end of the distraction phase (group 2 animal).

Fig 5a *(Left)* Whole sample from the group 1 animal (toluidine blue; original magnification ×5).

Fig 5b *(Right)* Whole sample from the group 2 animal (Goldner's trichrome; original magnification ×5).



between the screw and the oral environment. This screw was loose and partially unscrewed. Both the screw and the upper fragment of the distractor were removed. An area of resorption was observed on the transported bone fragment. Granulation tissue was removed from the area, which was irrigated with serum and sprayed with chlorhexidine. The tissues were resutured with resorbable 3-0 suture, and the 8-week consolidation period was completed.

A resorption in height of around 2 mm of the transported bone fragment was observed, but there was no lower prolapse of this fragment toward the distraction chamber. There was a rounding of the mesial and distal edges of the transported bone fragment. On the buccal surface of the distraction chamber, the tissue was similar in hardness to the basal bone, based on palpation, although with a slight depression compared with the cortical surface of the basal bone. Greater thickness of the cortical bone was observed on the lingual surface of the distraction chamber, with a lesser depression of the distraction chamber surface.

Histologic Study

The histologic study was performed independently by 2 operators using the more distal sections of the samples from the animals from groups 1 and 2 (Figs 5a and 5b). Table 1 displays the results for the qualitative histologic variables studied. Histologic assessment and description were individually performed for each animal in the study.

Group 1 Animal. At 40× magnification, the basal bone was mostly mature, type 2 lamellar cortical bone formed by central osteons and external and internal circumferential bone. New bone formation was observed at the level of the periosteum and endosteum; it thickened as it approached the regeneration chamber. It was observed in the upper third of the periosteum and, in the endosteum, in the upper half of the medullary cavity (Figs 6a and 6b).

At magnifications of 100× to 400×, most of the newly formed bone observed was immature woven bone characterized by the presence of osteoblasts with osteoid formation, a large number of rounded

osteocytes with highly visible nucleus and large lacunae, anarchic distribution of cells and extracellular matrices, architectural disorder of collagen fibers (polarized light), and numerous vessels. The extracellular matrix was intensely stained with toluidine blue, allowing differentiation between the more weakly stained mature bone and the cement line demarcating it. The neoformed bone showed formation of immature bone by intramembrane ossification, with no signs of chondrogenesis. In some areas of this newly formed bone, the extracellular matrix and reticular bone cells were arranged in a more organized manner around the vessels to produce parallel-fibered immature bone, with ellipsoidal osteocytes forming primary osteons or primary circumferential bone.

The medullary cavity of the basal bone was lengthened, communicating toward the distraction chamber. In the periosteal area, as the distraction chamber was approached, the osteoblast palisades were increasingly arranged in a lengthened manner and parallel to the distraction vector.

At ×40 magnification, the distraction chamber showed only trabecular bone. The osteotomy area was well defined, with a difference in staining between the native bone (weak) and newly formed bone (intense). The cement line separating the newly formed and native bone was also intensely stained.

Thin trabeculae were observed, covered with osteoblasts in a palisade arrangement, producing osteoids that filled and communicated throughout the chamber. Most of the trabeculae were arranged parallel to the distraction vector. There was an occasional isolated trabecula and a central area joined to the transported bone that appeared empty of bone. Only trabecular bone was observed. There was a greater density of trabeculae in the lingual area versus the buccal area, and communication with the medulla of the basal bone.

At magnifications of 100× to 400×, observation of the trabecula-free central area under polarized light revealed bundles of collagen fibers arranged parallel to the distraction vector (Figs 7a and 7b). The trabeculae consisted primarily of disorganized immature bone. There were also smaller areas of parallel-fibered bone.

At 40× magnification, distal sections of the transported bone fragment were observed to be largely formed of lamellar cortical bone with internal cavities of connective tissue. Newly formed bone was also observed in the lingual periosteal area and the basal bone.

At magnifications of 100× to 400×, in the transported segment, some fragments of necrotic bone appeared, characterized by osteocytic lacunae, anarchic morphology and arrangement, empty lacunae, areas of resorption with the presence of osteoclasts, and little birefringence of collagen fibers.

Remodeling osteons appeared in the necrotic areas with resorption phases (presence of osteoclasts) and formation phases (vessels, osteoblasts, and newly formed bone).

Group 2 Animal. At 40× magnification, the basal bone showed mostly lamellar cortical bone and a large amount of remodeling osteons. There was an elongation of the medulla toward the distraction chamber in a more demarcated manner than in the group 1 animal.

Table 1 Histologic Variables in the Distraction Chamber

	Group 1	Group 2	Group 3
Woven bone	> 75%	< 25%	< 25%
Parallel-fibered bone	< 25%	> 75%	< 25%
Lamellar bone	< 25%	< 25%	> 75%
Trabecular structure	> 75%	< 25%	< 25%
Cortical structure	< 25%	50% to 75%	> 75%
Continuity			
Lingual surface	50% to 75%	> 75%	> 75%
Buccal surface	< 25%	> 75%	> 75%

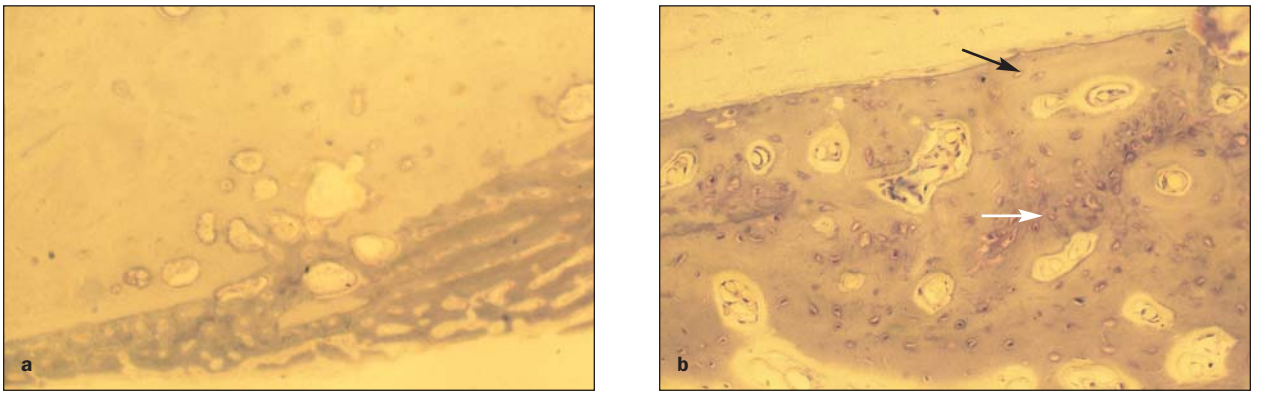


Fig 6 Lingual basal bone (group 1 animal). *(a)* Newly formed bone in the lingual periosteal area close to the distraction chamber (toluidine blue; original magnification ×40). *(b)* Woven bone *(white arrow)* and parallel-fibered bone *(black arrow)* were interwoven (toluidine blue; original magnification ×200).

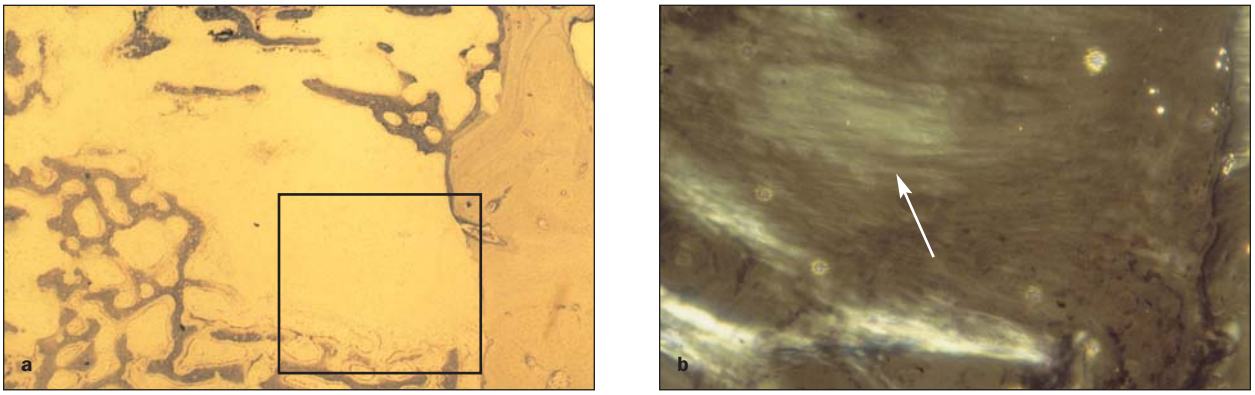


Fig 7 *(a)* Area without bone formation in the distraction chamber of the group 1 animal (toluidine blue; original magnification ×40). *(b)* A detail showing the collagenous fibers parallel to the distraction vector in the area without bone formation *(white arrow)* (original magnification ×200).

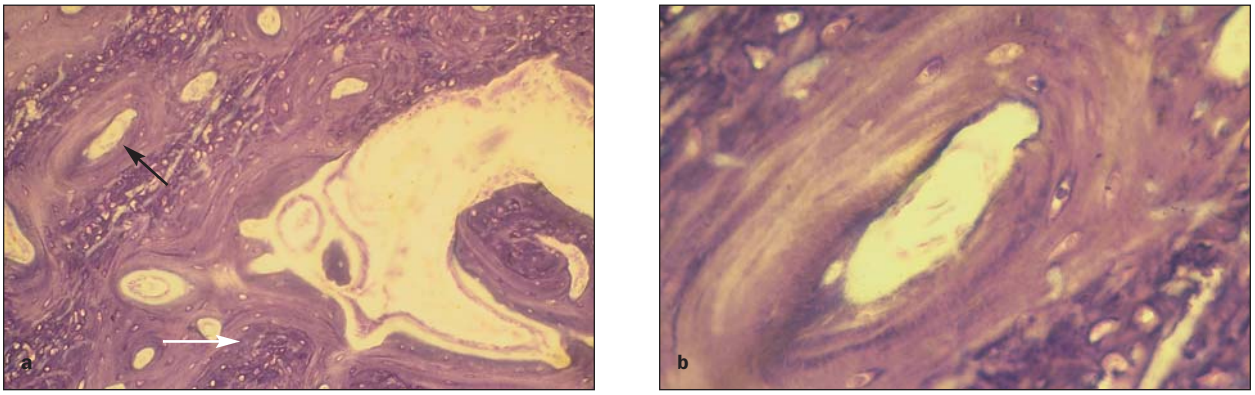


Fig 8 Newly formed bone in lingual area of the chamber (group 2 animal). *(a)* Woven bone *(white arrow)* and interwoven parallel-fibered bone *(black arrow)* (toluidine blue; original magnification ×100). *(b)* Primary osteon comprised of parallel-fibered bone. No inversion cement line was observed (toluidine blue; original magnification ×400).

Buccal and lingual periosteal new bone formation (thicker on the lingual side) appeared along the entire length of the basal bone but was greater in the apical third. There was formation of new endosteal bone only in the apical third. The distraction chamber was wider on the buccal side than on the lingual side. At magnifications of 100× to 400×, the newly formed periosteal bone was more organized than in the group 1 animal and was formed by circumferentially arranged parallel-fibered bone.

At a magnification of 40×, complete communication of the trabeculae was seen throughout the distraction chamber; this was not observed in the group 1 animal. Thick trabeculae were arranged in the lingual and buccal areas with a very similar morphology to that observed in the cortical or circumferential bone demarcating the central medullary cavity; this also was not observed in the group 1 animal.

At magnifications of 100× to 400×, newly formed bone was observed that was difficult to distinguish from native bone, although the former presented was more intensely stained. The arrangement of collagen fibers confirmed that it was largely parallel-fibered bone with interspersed areas of woven bone. Immature bone consisted primarily of osteons and primary trabeculae. No undulating cement lines were seen, indicating absence of osteoclastic activity (Figs 8a and 8b).

In the transported bone segment at 40×, isolated lamellar cortical bone was observed in the buccal and lingual areas. The distractor retention screw was located in a central area of immature bone.

Group 3 Animal (Control). At 40× magnification, lamellar cortical bone was seen to demarcate the medullary cavity, with scant areas of trabecular bone. The cross-sectional profile was more oval than the other samples studied. No new bone formation was observed in the periosteum or endosteum. The inferior dental canal was well-defined and rounded, unlike that of the animals from groups 1 and 2. Numerous remodeling osteons were observed.

Histomorphometric Study

Histomorphometric analysis was performed by a single operator on the same samples used in the histologic study. In the control animal, a single bone area density value was obtained for the entire sample,

Table 2 Bone Area Density and Bone Perimeter (Means ± SD)

	Group 1	Group 2	Group 3
Bone area density (%)			
A	66.58 ± 5.78	77.35 ± 6.67	—
B	36.61 ± 9.79	58.72 ± 8.30	—
C	77.29 ± 0.08	67.92 ± 2.77	71.45 ± 7.18
Bone perimeter (mm)			
A	196.30 ± 48.59	62.58 ± 11.54	—
B	262.89 ± 10.46	201.44 ± 22.64	—
C	176.42 ± 29.88	140.41 ± 43.05	200.27 ± 32.26

A = transported bone; B = distraction chamber; C = basal bone.

Table 3 Bone Height, Trabecular Width, and Osteoid Area Density

	Group 1	Group 2	Group 3
Bone height (mm)			
A	4.89 ± 0.45	2.75 ± 0.06	—
B	4.65 ± 0.06	6.21 ± 0.18	—
Total	21.31 ± 0.32	18.37 ± 0.50	15.72 ± 0.07
Trabecular width* (μm)			
Buccal	90.00 ± 4.24	154.50 ± 21.64	—
Lingual	134.00 ± 15.56	229.50 ± 29.24	—
Osteoid area density* (%)			
Buccal	3.75 ± 1.28	2.09 ± 0.79	0.79 ± 0.32
Lingual	4.08 ± 0.46	1.61 ± 0.33	0.70 ± 0.14

*Distraction chamber.
A = transported bone; B = distraction chamber; C = basal bone.

which was comparable to the results for area C for groups 1 and 2. Trabecular width was not determined in the control animal because the bone was mostly cortical. Means and standard deviations for each of the variables examined are exhibited in Tables 2 and 3.

DISCUSSION

There have been few studies of experimental alveolar distraction,[3,16,22,23,37,41,42] most of which used beagle or mongrel dogs as a biomodel. Differences in the distractor model and distraction protocols used hamper comparison of the clinical results of these studies. However, distraction is always produced in the same way, facilitating comparison of histologic and/or histomorphometric findings.

The present study included a group with a short consolidation period (4 weeks), bearing in mind that a consolidation time of 8 to 12 weeks has been applied in most clinical studies.[27,29,30,32,34,35,43] Various authors have proposed the reduction or elimination of the consolidation period.[16,22,44]

An extrabone design was chosen because some studies have demonstrated that intrabone distractors require a longer consolidation period to allow bone regeneration between withdrawal of the distractor and placement of the implants,[27,31,45] because it is unlikely that the bed left after removal of the distractor would be ideal for implant placement. In addition, a greater number of complications related to stability and incorrect distraction vectors have been reported with intrabone distractors versus extrabone distractors.[27,31,35,43,46]

The present study demonstrated that when the bone fragment was prematurely exposed during the latency period, it was not possible to achieve a new covering with soft tissue, despite curettage, cleaning, and resuturing. This may be the result of necrosis in the transported bone fragment produced by the absence of mucosal covering and the resulting inadequate vascularization. In clinical studies, dehiscences have been successfully treated with local procedures.[6,34,35,47]

Until recently, because of the lack of knowledge of the characteristics of immature bone, many studies failed to distinguish between the 2 types of immature bone. Thus, woven or reticular bone was considered immature bone, and the proportion of parallel fibered or woven bone, which appears to have different biomechanical properties,[48] was not identified. In addition, histomorphometric studies can show major intra- and interobserver differences, compromising the objective comparison of results.[49]

The arrangement of trabeculae was parallel to the distraction vector in most of the sections from the group 1 animal, but was only occasionally so in samples from the group 2 animal. Moreover, collagen fibers arranged along the distraction vector were only observed in a localized manner in areas of the group 1 animal where bone had not been formed. It appears that the parallel arrangement of collagen fibers and the appearance of fibroblastlike fusiform cells (typical of the distraction microenvironment) are mostly observed during the distraction phase and the first few weeks of the consolidation period.[12,50]

The regenerated bone in the group 1 animal showed the characteristics of woven bone, with a largely trabecular arrangement, whereas that of the group 2 animal was mainly composed of parallel-fibered bone, with a more corticalized arrangement. Mature lamellar bone presence was not observed in the distraction chamber in any of the samples; it has only been observed after the third month of consolidation in other experimental studies.[37] To date, no schedule of implant placement has been proposed based on type of bone (density) or the percentage of mineralized bone in bone created by distraction.

The bone density of the chamber in the group 1 animal (36.61% ± 9.79%), largely formed by woven bone, was similar to those reported by Zaffe and associates[28,44] in human samples; they presented an area density of 50% after eight weeks of consolidation. In the same study, the area density was reduced to 37% at 12 weeks because of osteoclastic activity.

Oda and associates, using a dog model, shortened the consolidation period and placed implants immediately after the distraction. They observed a bone-implant bond in all cases, with a bone-implant contact of 30.2% in the distracted area.[22] The area density at 8 weeks after implant placement (39.3% ± 10.9%) was lower than that observed in the group 2 animal (58.72% ± 8.30%) of the present study; this may have been the result of damage suffered by the regenerated bone during implant bed preparation. At that time (8 weeks), the chamber showed characteristics of immature bone with a predominance of parallel-fibered bone, as observed in the present study.

Nosaka and associates reported the osseointegration of implants placed in the alveolar ridge after horizontal distraction after only 12 days of consolidation.[51] Implants have also become osseointegrated in bone regenerated by mandibular elongation distraction after only 3 weeks of consolidation.[16] In the present study, the samples with the longer consolidation time (8 weeks) showed no signs of remodeling or presence of lamellar bone in the distraction chamber. Oda and associates[22] and Nosaka and colleagues[51] reported the appearance of lamellar bone at 12 to 14 weeks of consolidation.

As reported in other studies of alveolar distraction,[22] greater bone density, maturation, and bone organization were seen in the lingual versus the buccal area of the distraction chamber. This difference was corroborated by the histomorphometric data in this study. Osteoblastic activity, represented by osteoid area density, was higher both buccally and lingually in the group 1 animal than in the group 2 animal and was least active in the control animal. An increase in osteoid production was also reported by Gaggl and colleagues[37] in a histologic evaluation of sheep after 2 months of consolidation.

The transported bone fragment undergoes an intense remodeling process, evidenced by the increase in remodeling osteons.[46] In the group 2 animal, the lamellar cortical transported bone showed a less intense remodeling and a greater height resorption compared with the group 1 animal. The only areas with bone regeneration were those close to the occlusal fixation screw, which was prematurely withdrawn. This is consistent with the histomorphometric data, which showed greater porosity (ie, perimeter) (196.30 ± 48.59 mm versus 62.58 ±11.54 mm) and height (4.89 ± 0.45 mm versus 2.75 ± 0.06 mm) of the transported bone in the group 1 animal versus the group 2 animal. These data may indicate that implant placement at 4 rather than 8 weeks of consolidation can help avoid resorption of the transported bone fragment. The morphometric results for the total height attained show that more height was gained in the group 1 animal than in the group 2 animal. In the study by Oda and colleagues,[22] of the vertical height obtained initially, only 0.72 mm had been lost at 12 weeks of consolidation. This loss may have been so small because of the early implant placement. Taking into account the limited sample size of the present study, the results obtained support the hypothesis proposed by Zaffe and associates,[44] Oda and colleagues,[22,23] Nosaka and coworkers,[16,51] and Raghoebar and associates[47] that the best time for implant placement is probably after 2 to 4 weeks of consolidation for the beagle dog mandibular model and, by extrapolation, after 6 to 8 weeks of consolidation for humans. The consolidation period can be even shorter if the implants are not loaded immediately. However, these novel protocols require further evaluation of different consolidation periods in study designs that include the placement of implants.

CONCLUSION

This preliminary study proposes a methodology for the experimental histologic study of alveolar distraction. In the present application of this approach, histologic and histomorphometric differences were found between the 4-week and 8-week consolidation periods. These findings need to be corroborated by experimental studies with larger samples and by prospective clinical studies.

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