Evaluation of minipigs as an animal model for alveolar distraction

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Objective. Alveolar distraction osteogenesis is a relatively novel tissue regeneration technique that remains, in some respects, at an experimental stage. The selection of an experimental animal biomodel is not clearly defined, with reports in the reviewed literature on dogs, sheep, and monkeys. The present objective was to compare 2 experimental biomodels (dog and minipig) using an alveolar distraction protocol with a novel prototype distractor.

Study design. Three beagle dogs and 3 Göttingen minipigs were evaluated, placing the distractor at the right hemimandible alveolar ridge after previous extraction of premolars. The radiological examination was carried out immediately after the distraction and at 2 weeks of consolidation.

Results. Satisfactory clinical and radiological results were obtained in 2 of 3 beagle dogs after the previous unilateral extraction of mandibular and maxillary premolars. During the consolidation, a height gain of approximately 5 mm was observed, with the appearance of radiodense bone trabeculae in the distraction chamber. The distraction failed in all of the minipigs.

Conclusion. The minipig was not an appropriate biomodel for the study of alveolar distraction because the investigators were not able to maintain the seal of the distraction chamber or the stability of the distractor.


Distraction osteogenesis is a process by which bone is lengthened or heightened by creation of an osteotomy so that the gap between them gradually increases with no interruption of the blood flow. This process is based on the so-called Ilizarov effects: (1) gradual traction of the tissues creates stress that can activate 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 flow.1,2

Vertical augmentation of the mandibular or maxillary alveolar ridge to increase bone volume for implants has shown variable and controversial outcomes in comparison with horizontal augmentation. Different techniques have been used to achieve vertical alveolar augmentation, including onlay or inlay grafts, guided tissue regeneration, sinus floor grafting, and transposition or lateralization of the inferior dental nerve. The alveolar distraction technique appears to produce more rapid, predictable, and permanent outcomes compared with other regeneration techniques, according to initial reports of its clinical application.3,4 However, this technique is not free of certain complications, which have been reported in both experimental and clinical studies.5

A biological model for experimental alveolar distraction must offer an adequate amount of bone for the placement and functioning of distraction devices designed for clinical use in humans. Mandibular elongation by distraction has been developed in small animals at the lower end of the phylogenetic scale (rat, rabbit) and also in more evolved animals (minipig, dog, sheep, and primate). Nonhuman primates undoubtedly represent the ideal biological model for research but their high cost is generally prohibitive. Good outcomes have been reported for alveolar distraction in primates,6 sheep,7 and dogs.8-10 There has only been 1 study of alveolar distraction in minipigs11 using a horizontal alveolar distractor in the maxilla, but no study has used the minipig for vertical alveolar distraction, despite the close similarity between the bone remodeling rate of pigs and that of humans.12 With this background, our group decided to evaluate whether the minipig is an adequate biological model for vertical alveolar distraction in comparison with other animals in which satisfactory outcomes have been obtained. The present study aimed to establish whether the minipig or beagle offered the

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most appropriate animal model, at the same anatomic localization, for the development and maintenance of bone regenerated by alveolar distraction using the same novel alveolar distractor. The in vivo viability of this distractor was also tested.

**MATERIAL AND METHODS**

This study was carried out at the Department of Medicine and Experimental Surgery of "Gómez Ulla" Central Military Hospital in Madrid. Three adult male Göttingen minipigs and 3 adult male beagle dogs were used. The handling of the animals adhered to established local norms for the protection of experimental animals, and the study was approved by the Ethical Board of Animal Investigations of the hospital. The preoperative and postoperative care of these animals was overseen by veterinarians to ensure proper treatment. The animals were fed with a soft diet to reduce mechanical interference with wound healing after surgery.

The prototype alveolar distractor was designed by the authors and made by Impladent (Impladent SL, Barcelona, Spain) (Fig 1). The maximum distraction that can be achieved with this distractor is 6.6 mm (10 turns) before the upper and lower fragments separate. It is an extrabone device made of grade 5 titanium. It has a 12-mm-long distraction body, lower anchor plate with 2 holes, and upper anchor plate with 1 hole. A screw of 6.8 mm length and 1.7 mm diameter is inserted into each hole. Each turn of the distraction screw produces a height gain of 0.66 mm. It is designed to move small bone fragments and is useful to regenerate alveolar defects between adjacent teeth.

The surgical equipment included a micromotor for surgery and implantology (Controller Set, Nobel Biocare, Göteborg, Sweden) and Lindeman burs (diameter 1 mm, length 10 mm) (Komet, Gebr Brasseler, Lemgo, Germany) to form the bone holes for the retention screws (Komet, Gebr Brasseler, Lemgo, Germany). The osteotomies were performed by means of an oscillating saw with 32 mm × 14 mm × 10 mm blades (OMS 5000, Nouvag, Gouldach, Switzerland).

A radiographic positioner made of bite-blocks (Stable Bite-Block, Dentsply-Rinn, Elgin, USA), galvanized wire (diameter 1.7 mm), and autopolymerizable acrylic resin was used. Intraoral radiographies were taken (Ultra-speed D, Kodak, Stuttgart, Germany) with a long cone apparatus (Oralix 65 S, Philips, Milan, Italy) that emitted at 65 kilovolt (kv) and 7.5 milliamperes (ma). The radiographs were performed using parallel technique for an exposure time of 34 milliseconds (msec) and were developed with an automatic developer (Dent X 9000, Philips, Eindhoven, Holland) for 4.5 minutes at constant temperature of 28.1°C using fresh developing and fixing liquids (RP X-OMAT, Kodak, Stuttgart, Germany).

The induction of anesthesia in minipigs was performed with carazolol (0.2 mL/10 kg), azaperone (0.25 mL/10 kg), atropine (0.5 mL/10 kg), and midazolam (1 mL/10 kg); the anesthesia was maintained with 2% isoflurane plus N₂O/O₂ and reversed with flumazenil (10 mL/50 kg). In beagles, the induction used was medetomidine (20-40 mg/kg), butorphanol (0.2-0.4 mg/kg), and atropine (0.5 mL/10 kg); the anesthesia was maintained with 2% isoflurane plus N₂O/O₂ and reversed with atipamezole (20-40 mg/kg). Both biomodels underwent a 3-day course of antibiotic and anti-inflammatory treatment, using streptomycin + penicillin G + dexamethasone (minipigs: 5 mL/kg/animal/day; beagles: 2 mL/kg/animal/day) and flunixin (minipigs: 3 mL/kg/animal/day; beagles: 1 mL/kg/animal/day).

All surgical procedures were performed by the same surgeon, and the same anatomic site and surgical approach were selected in both species. In the dogs, 4 mandibular premolars and 3 maxillary premolars were first extracted on the right side, and, 3 months later in a second phase, the distractor was placed on the alveolar ridge distal to the right mandibular canine (Fig 2). A full thickness mucoperiostal incision was performed between the canine and first molar, and the mucosa was reflected, exposing the lateral surface of the mandible. A horizontal osteotomy was made with an oscillating saw (OMS 5000, Nouvag, Gouldach, Switzerland) at 5 mm from the upper edge of the ridge. The distractor was positioned and 6-mm long bone holes were performed with a Lindeman bur (fissure bur) (Komet). Retention screws were placed in the following order: upper-basal, occlusal, and then...
lower-basal. Profuse irrigation with sterile saline was always used. Immediately afterwards, 2 osteotomies were made with a fissure bur on the upper side of the transported bone segment, 20 mm apart. These osteotomies are useful to avoid cutting edges on the transported bone when it is distracted.

Finally, 2 vertical osteotomies were performed with an oscillating saw (OMS 5000, Nouvag) on previous marked locations with fissure bur, avoiding injury of the lingual mucosa (Fig 3). The mobility of the transport bone segment was confirmed, and the distraction was tested (Fig 4). The wound was sutured by interrupted suture with 3-0 nylon but the top of the distractor was left uncovered to provide access during the distraction period.

The same distraction protocol was followed in both biomodels with a latency period of 7 days and distraction rate of 1 mm/day/5 days (one and a half turns per day). Distraction was performed under sedation using an intramuscular dose of medetomidine (20-40 mg/kg) and butorphanol (0.2-0.4 mg/kg). When there was a clinical evolution compatible with bone regeneration, radiographs were made immediately after the distraction and at 2 weeks of consolidation. Between the different phases, the animals were kept in cages containing 2 animals each.

RESULTS

Dehiscence and contamination of the distraction area were observed in 2 minipigs during the latency phase of distraction, exposing the distracted bone fragment to the oral medium and producing inflammation and mobility incompatible with the bone regeneration of the distraction chamber (Fig 5). Curettage, cleaning with serum, and resuturing of the area were carried out on successive days, but the wound continued to be exposed and contaminated. The distractor was then removed, followed by curettage and suturing of the area. We consider that the constant rooting and biting movements made by minipigs compromise the maintenance of the soft tissues that protect the distraction. Dehiscence and contamination were observed in the other minipig 2 days after the beginning of distraction with the same result, although the distraction area was again covered by mucosa during the latency phase.

In one of the beagle biomodels, dehiscence and contamination of the distraction area were observed during the distraction phase. Attempts were made to protect the distraction chamber with repeated curettage and suturing of the area, without obtaining satisfactory results. The distractor was then removed.

In the other 2 dogs, distraction of the alveolar segment was performed with no complications. A good clinical stability of the regenerated area and adequate soft tissue covering were achieved, until 2 weeks of consolidation (Fig 6).

In the clinical evaluation, a 5-mm vertical augmentation was observed both in the hard tissues and in the attached mucosa on the transport segment. During the distraction phase, no resistance was observed during
the first 2 activation days but resistance increased between the third and fifth days. This may have been caused by a greater strain on the attached mucosa.

The radiographic study at the end of the distraction showed the size of regeneration chamber obtained (width 20 mm, height 5 mm) and the complete radiotransparency of the whole surface, with clear demarcation of the distraction chamber (Fig 7, A). After 2 weeks of consolidation, a rounding of the sharp edges of the distracted fragment could be observed, with radiodense trabeculae visible from the basal bone and transport bone toward the central zone of the chamber, and some “in-island” trabeculae. The demarcation of the regeneration chamber was preserved. A slight resorption was observed around the upper anchor screw, due to the exposure of this screw to the oral medium and the appearance of mucositis (Fig 7, B).

DISCUSSION

As reported by other authors, we found that the previous extraction of premolars and an adequate time of healing (12 weeks) are essential to obtain an edentulous alveolar ridge with organized alveolar bone and healed and stable soft tissues for distractor placement. The unilateral mandibular placement of a distractor has shown satisfactory outcomes in dogs without teeth or with teeth extracted in the opposing arch and also in sheep, while a successful bilateral placement has been reported in monkeys.

Mucosal complications have been reported in dogs at 12 days after the distraction, which were treated by the immediate removal of the distractor and the placement of implants. There have been frequent clinical reports of mucosal complications, which were readily resolved with local treatment. We failed to obtain satisfactory results in the minipig, as also reported by Henkel et al, who observed mucosal complications in 9 out of 15 pigs studied, with a resulting loss of the distractor. We consider the mandible a less favorable site than the maxilla in this biomodel. On the other hand, vertical distraction is not feasible in the maxilla of the minipig, due to its anatomical structure.

We consider the stability of the bone fragment—distractor complex to be indispensable for the performance and maintenance of the distraction. The distractor used provides adequate stability with 3 anchoring screws, and only the minipig biomodel showed losses of the upper fragment of the distractor through the unscrewing of the internal distraction screw. Oda et al produced distraction with the insertion of implants in one study and using a prototype distractor in another, although neither system gave adequate stability to maintain the regeneration chamber during consolidation. They achieved the necessary stability by circummandibular suturing in the
latency period, by an adequate covering and closure of the soft tissues, and by placing the implants immediately after completion of the distraction procedure. Cases that showed any dehiscence were treated with topical chlorhexidine and the distraction was halted until reepithelization occurred. We consider this to be a good option when the dehiscence has not produced invasion of the distraction chamber by soft tissues. Block et al.\footnote{12,17} were able to achieve an excellent stability of the complex by anchoring their prototype to 4 previously osseointegrated implants. They reported no dehiscence or wound opening in any of the 8 dogs treated. However, their prototype is not viable for use in humans.

In both biomodels used in the present study, if the bone fragment was prematurely exposed in the latency period, a covering with soft tissue could not be achieved, despite curettage, cleaning, and resuturing procedures. When exposure was observed, we changed the type of suture, combining simple suture, cross suture, or modified mattress suture with 3-0 nylon. However, the dehiscence of the wound and exposure of the mobilized fragment always recurrent. A migration of epithelial tissue was produced in the distraction chamber, making bone regeneration nonviable. This migration may be caused by necrosis in the transport bone due to the lack of mucosal covering and the inadequate vascularization. We consider a correct suturing with a complete covering of the occlusal anchoring to be essential for isolating the distraction chamber. Wounds sutured with nylon and a triangular needle were more closed and less contaminated compared with those in which silk or resorbable suture (Vicryl, Ethican, Brussels, Belgium) were used.

Interpretation of experimental studies must take account the great differences between humans and species of lower phylogenetic evolution. Each animal species has distinct regeneration and remodeling times. There is an inverse proportional relationship between the rate of bone regeneration and the phylogenetic evolution of the animal. Thus, each remodeling cycle (denominated sigma) lasts 3-6 months in humans, 3 months in dogs, and 6 weeks in rabbits.\footnote{15} Rabbit and rat biomodel results should, therefore, be interpreted with greater caution than those from studies of dogs, sheep, minipigs, or primates. Stewart et al.\footnote{16} found no differences in the mandibular elongation of rabbits between the use of a slow (0.5 mm/twice daily/15 days) versus rapid (1.5 mm/twice daily/15 days) distraction rate, in contrast to the differences found in more evolved animals, such as minipigs and sheep.\footnote{12,17}

Our radiological findings in the 2 beagle dogs are comparable with those reported by other authors who performed alveolar distraction in dog mandible,\footnote{8-10} who also reported the rounding of edges and a vertical resorption of approximately 1 mm of the transport fragment. In the dog model, the limits of the distraction chamber cannot be distinguished from the adjacent bones at 8 weeks of consolidation and the radiodensity is homogeneous.\footnote{9,10} In humans, this radiological homogeneity of the distracted area has been observed in maxillary alveolar distraction at 12 weeks of consolidation.\footnote{3,18}

In a study of alveolar distraction in sheep mandible, a slight radiodensity of the distraction gap and a fading of the boundary with the distraction fragments were observed at 1 month of consolidation. At 2 months, the radioopacity was reduced but there were still differences between the gap and the transport and basal bone. At 3 months, the gap area showed a similar density to the adjacent upper and lower bone, although it could still be distinguished. At 6 months, a radiologically homogeneous area was observed and the gap could not be distinguished.

Cope and Samchukov\footnote{19} described distinct radiological patterns of mineralization in a dog mandibular elongation model. At 2 weeks of consolidation, 54% of samples presented ossification and mineralization signs, with slender trabeculae joined to the original bone and “in-island” trabeculae in the central zone of the gap, corresponding to the pattern observed in our alveolar model. The remaining 46% of samples presented mineralization signs, with 2 well-defined areas joined to the original bone and a central radiotransparent interzone.

At 4 weeks, 69% of the samples presented 2 regeneration areas and a central radiotransparent interzone, whereas the remaining 31% showed a continuous mineralization area with no central interzone. At 8 weeks, the samples presented greater density; 50% of the samples still had a central radiotransparent interzone, whereas 25% presented uniform mineralization of the gap and corticalization of the regenerated bone, always starting from the medial margin. It is considered that 30% to 60% of bone mineralization can be gained or lost without being radiologically observed, so that the radiotransparent interzone may have a high percentage of mineralization that is not detected. In all consolidation periods observed, the clinical stability of the regenerated area was directly related to the presence of a central interzone and to the width of the regenerated area in relation to the width of the original bone.

At any rate, it is difficult to extrapolate the radiological pattern when the distraction is parallel to the longitudinal axis of the bone (eg, mandibular elongation) compared with distraction transversal to this axis (eg, vertical alveolar mandibular distraction).

CONCLUSIONS
The minipig is not an appropriate biomodel for the study of alveolar distraction, because it is not possible
to maintain the seal of the distraction chamber or the stability of the distractor.

The hemimandible of the beagle dog, with a period of healing after the unilateral extraction of mandibular and maxillary premolars, is an appropriate biomodel and anatomical location for alveolar distraction osteogenesis.

It was clinically and radiologically demonstrated that this novel distractor prototype can achieve and maintain bone regeneration in the mandible at 2 weeks of consolidation in a beagle dog biomodel.

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