Published in final edited form as:

Arch Oral Biol. 2009 March ; 54(3): 205–215. doi:10.1016/j.archoralbio.2008.12.002.

The effect of periosteal injury and masticatory micromovement on the healing of a mandibular distraction osteogenesis site

Zongyang Sun^{1,2,*} and Susan W. Herring¹

¹ Departments of Orthodontics and Oral Biology, School of Dentistry, University of Washington, Seattle, WA

Abstract

Objectives—Surgical periosteal injury and masticatory loading are likely factors affecting the healing of a mandibular DO site. This study is aimed to characterize the healing features of an early-phase mandibular DO site and assess the effects of these factors.

Design—Eighteen 3–6 month-old miniature pigs received a right mandibular osteotomy and were distracted for 5 days (1 mm/day) and consolidated for 0, 1 or 2 weeks (Groups A, B and C, respectively). Bone formation, chondrogenesis and vascular structure of the distraction site were measured using histological methods and their changes with consolidation time were characterized. The effect of periosteal injury was assessed by comparing the more severely injured lateral side with the less disturbed medial side. The effect of masticatory loading was evaluated by relating the healing features to the interfragmentary micromovement caused by soft-diet mastication.

Results—With consolidation time, bone formation and chondrogenesis became stronger while vascular structure became more mature. Compared to the medial side, bone formation and chondrogenesis on the lateral side were significantly delayed in Groups A and B, but not in Group C, in which periosteal recovery had occurred. No difference was found for vascular measurements between the medial and lateral sides. In Group B, bone formation, but not chondrogenesis or vascular structures, tended to be negatively correlated with the magnitude of masticatory micromovement during the distraction phase.

Conclusion—The results suggest that periosteal injury inhibits early mandibular DO site healing, whereas micromovement from soft-diet mastication mechanics has a negligible effect.

Keywords

Distraction osteogenesis; bone formation; cartilage; vasculature; micromovement

INTRODUCTION

Ever since its first clinical application in the early 1990's (1), distraction osteogenesis (DO) has become a popular surgical alternative to treat severe mandibular hypoplasia or retrognathia. Because it is a mechanically induced bone lengthening process through a so-called tension-stress mechanism (2,3), the last two decades have seen extensive attention being paid to the

*Corresponding author: Division of Orthodontics, College of Dentistry, The Ohio State University, 305 W 12th Ave, Rm 4088 Postle Hall, Columbus, OH 43210, Tel: 1-614-247-7944; Fax: 1-614-688-3077; Email: sun.254@osu.edu.

²Current address: Division of Orthodontics, College of Dentistry, The Ohio State University, Columbus, OH

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

distraction mechanics itself (4–11). Based on these studies, some recommendations for optimal distraction protocol were recently proposed (12).

To optimize the DO process, however, other affecting factors also need to be considered. Preservation of soft tissue, especially the periosteum, is considered critical for limb bones (13). Histological and molecular evidence also points to a significant contribution of the periosteum in DO site bone formation (14–16). For a mandibular DO site, injury to the periosteum is unavoidable during the surgical procedures of osteotomy and placement of the distractor, although the severity of injury changes with many factors, such as surgical methods and the choice of distractors. For extraoral distractors, the lateral mandibular side is often selected as the access route for osteotomy and the location for distractor placement, which results in more damage to the lateral than to the medial periosteum. The effect of such periosteal injuries on the DO site healing, however, has not been systematically investigated.

Stability of the distraction site is another potential factor that may influence healing. Rigid fixation of the distractor has been considered crucial for DO site bone formation (13,17). Device instability could affect the DO site in at least two ways, by increasing chondrogenesis (rather than osteogenesis) (13,18) and by disrupting angiogenesis and vascularization (19). Although gross instability is damaging, micromovement (<1 mm in long bones) of a stably fixed fracture or distraction site has been found to be stimulatory (20–22). For the mandible, complete stabilization of the osteotomy is not possible if masticatory activity occurs during the early phase of distraction osteogenesis, which in fact is often recommended by surgeons to prevent TMJ ankylosis. Our previous study found that in the pig mandibular DO model, soft-diet mastication caused micromovement across the DO site with an average peak of 0.3 mm during the distraction phase (23). Although this micromovement gradually diminished to the control level after 1 week of consolidation, it may have had an effect on DO site healing.

The overall purpose of this study, therefore, is to characterize the features of an early-phase healing mandibular DO site and assess the effects of periosteal injury and masticatory micromovement. The periosteal effect is examined by comparing the less-damaged medial and more-damaged lateral sides, while the masticatory effect is examined by relating the repair process to micromovement measured in vivo in the same animals (23). Three tightly interrelated features are used to assess the mandibular DO site healing, bone, cartilage and vasculature. Specifically, we ask three questions. One, how do these features change with increasing consolidation time? Two, does periosteal injury adversely affect these features, and if it does, for how long? Three, does masticatory micromovement of the distraction site affect these features?

MATERIALS AND METHODS

Animals

The sample consisted of 18 Hanford strain miniature pigs (3–6 months and 10–30 Kg) of either sex, all derived from the previous study on in vivo mechanics (23). All pigs received a unilateral mandibular osteotomy, followed by a DO protocol (0–1 day latency, 5-day distraction at 1 mm/day and then consolidation). Based on the duration of consolidation, the animals were divided into 3 groups (Group A, 0-week consolidation; Group B, 1-week consolidation; Group C, 2-week consolidation). Masticatory micromovement of the DO site was recorded daily from the superior and inferior aspects of the DO site (Fig. 1). Procedures and results for surgery, instrumentation and mechanical measurement have been presented previously (23). As detailed below, fluorescent vital bone markers were administered to assess bone apposition. All experimental procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Vital staining, specimen collection and processing

With the pigs under general anesthesia with isoflurane, fluorescent dyes calcein and alizarin complexone were injected intravenously (40 mg/Kg dissolved in sterile 0.9% saline and filtered at 0.22 μ m) 4 and 1 days, respectively, before sacrifice. On the terminal day, after mechanical data were collected (23), the head was perfused through the carotid arteries with 3 liters of heparinized 0.9% saline followed by 3 liters of fixative (Prefer, Anatech LTD, Battle Creek, MI).

After the head was skinned, the distraction site was removed from the mandible with approximately 1 cm of original bone on both the proximal and distal sides with the distractor still in place to hold the fragments as they were before sacrifice. This specimen was further fixed in Prefer for 2 weeks. Then, the specimen was cut into 5 blocks based on location (Fig. 1A); all cuts were parallel to the activating rod of the distractor. The most superior (above the distractor) and the most inferior (below the distractor) blocks were processed without decalcification. The blocks immediately superior and inferior to the distractor were decalcified. The middle block (underneath the distractor) was processed without decalcification in 8 pigs (A1–A4, B1–B4) and with decalcification in 10 pigs (A5, B5–8, C1–C5).

Uncalcified specimens were dehydrated in an ethanol series and infiltrated with MicroBed resin solution (Electron Microscopy Sciences, Fort Washington, PA). The resin was then cured using 365 nm ultraviolet light for 3–4 days. Embedded specimens were cut into sections parallel to the distractor at a thickness of approximately 30 μ m using a diamond circular saw (Leica SP 1600, Germany). Sections were flattened, dried overnight at 37°, and mounted on slides.

Decalcification was performed with 10% ethylenediamine tetraacetic acid (EDTA) or a mixture of 15% formic acid and 2.6% sodium formate for 3–4 months. After dehydration, decalcified specimens were embedded in paraffin and cut into serial sections parallel to the distractor 5–7 μ m in thickness.

Assessment of bone formation

Uncalcified sections were viewed under a fluorescence light microscope (Nikon Eclipse E400, Japan). For each specimen block, two sections were chosen to analyze mineralization of both the gap callus (inside the distraction gap) and periosteal callus (on lateral and medial bone surfaces). For gap callus, images were captured from the medial, interior and lateral regions of each distraction gap using the old bone cortices to establish the boundaries (Fig. 1B). For periosteal callus, images were captured from the medial and lateral bone surfaces for 3.5 mm on either side of the distraction site (Fig. 1B). All images were captured using a Spot camera (Spot image, Chantilly, VA) and encoded with randomly-generated file names to blind the analysis.

Two measurements were taken for these undecalcified sections with MetaVue software (Universal Imaging Corp, Downingtown, PA). One was mineral apposition rate (MAR), defined as the average daily distance between the fronts of the two fluorescent vital labels (calcein, green; alizarin complexone, red) (Fig. 2A). The other was mineral advancement, defined as the average distance from the old bone margin to the alizarin-labeled callus front (Fig. 2A).

Decalcified sections stained with hematoxylin-eosin (H&E) were used for general histology and measurement of callus bone volume fraction (BV/TV). Image capture was performed similarly as for undecalcified sections. BV/TV measurement was carried out in MetaVue. A 25×19 square grid was superimposed on each image, and BV/TV was calculated by dividing the number of intersections that landed on callus bone by the number of intersections on the whole periosteal area (periosteal callus BV/TV), or on the entire distraction gap excluding any tooth structure (gap callus BV/TV) (Fig. 2B).

Assessment of chondrogenesis

Alcian blue staining and immunohistochemical staining for collagen type II (Col-II), both employing decalcified sections, were used to assess chondrogenesis. For the latter technique, sections were deparaffinized, rehydrated, and endogenous peroxidase activity was blocked with 2% hydrogen peroxide. Antigen retrieval was performed with pepsin (Lab Vision Corporation, Fremont, CA) incubation at 37° for 10 minutes. After blocking of endogenous biotin and avidin (Avidin/Biotin blocking kit, Vector Laboratories, Inc., Burlingame, CA), sections were incubated with a mouse monoclonal Col-II antibody (working concentration 4 µg/ml, Lab Vision Corporation) at 4° overnight. Negative control sections were reacted to mouse IgG isotype (Dako Corporation, Carpinteria, CA). Sections were then reacted with biotin-conjugated anti-mouse secondary antibody, followed by avidin-labeled peroxidase (standard ABC kit, Vector Laboratories). Chromogen was developed with 3,3'-diaminobenzidine (DAB) (Vector Laboratories) during a 10–15 minute incubation. Sections were counterstained with Gill's Hematoxylin and cover-slipped. Alcian blue and immunohistochemistry gave similar results. Because the Col II reaction is presumably more specific than alcian blue, these sections were used for quantification.

Col-II reactivity was quantified as the linear percentage of Col II (+) margin, which was defined as the length of the distraction gap margin covering Col-II (+) matrix divided by the total length of the gap margins (Fig. 2C). This measurement was used to indicate the percentage of chondrogenic gap margins. Areas were not measured because cartilage matrix was typically only seen near the margins and because the boundaries of positive areas were indistinct.

Analysis of vascular structure

Lectin immunohistochemistry was used to identify endothelial cells (24,25). After blocking of non-specific binding as described above for Col-II, sections were incubated with unconjugated *Griffonia simplicifolia* lectin I (CSL I, Vector Laboratories) overnight. Negative control sections were reacted to PBS instead of lectin. The sections were then treated with biotin-conjugated secondary antibody, followed by incubation with avidin-labeled peroxidase and finished with chromogen development and counterstaining as described above.

Images of the distraction gap of stained sections were captured. Captured images were first color-thresholded to differentiate lectin-bound areas (endothelial cells) from the background. Because of the variation among sections in background staining, a unique threshold level was established for each section by excluding the color of osteocytes on two randomly selected images from that section. After application of the threshold, the gap site of interest was delineated by excluding bone matrix, marrow, tooth structures and artificial spaces (Fig. 2D). Two parameters were measured. Endothelial cell density was defined as the percentage of thresholded pixel area relative to the total area of the site of interest. Blood vessel lumen density was calculated first by defining blood vessels as all visible lumens having (1) cell and basement membrane linings, (2) thresholded pixels (e.g., endothelial cells) in the walls and (3) lumen area no smaller than 40 µm² (26), then by dividing the area of vessel lumens by the total area of the site of interest. The measurements for each DO gap region (medial, interior and lateral) were calculated by combining measurement of all images from that region.

Statistical analysis

Change with the increase of consolidation time was assessed by one-way ANOVA. The effect of periosteal injury was evaluated by comparing measurements from the medial and lateral regions using paired t-tests. The effect of masticatory mechanics was examined by one-way ANOVA between locations of high (superior and inferior) and low (interior) micromovement and by Pearson correlations between micromovement and histological measurements.

RESULTS

Three animals (A5, B3, B6) had distractor failures, and the osteotomies of these pigs were only minimally (<2 mm) distracted (23). Because of this, these animals were excluded from the analyses.

1. Change with consolidation time

1. 1 Bone—Typical periosteal callus formation is illustrated in Fig. 2A&B. Qualitatively, callus was trabecular and bone spicules were roughly perpendicular to the cortical surface. Measurements of periosteal callus are shown in Table 1. Comparison among groups (one-way ANOVA) found that with increasing consolidation time, mineral advancement and BV/TV measurements increased, usually significantly. Mineral apposition rate, however, typically peaked after 1 week of consolidation (Group B).

Examples of callus formation in the distraction gap are shown in Fig. 3. Callus in the medial and lateral regions of the distraction gap mainly arose from the cortex, either directly or indirectly from the approaching periosteal callus. Callus in the interior region, on the other hand, could arise from old trabecular bone or from periosteal callus. The orientation of trabeculae in the gap callus was mainly parallel to the vector of the distraction force, although randomly oriented callus was not uncommon.

Measurements of distraction gap callus are shown in Table 2. Generally, compared to the surface callus, gap callus grew at a slower pace (lower mineral apposition rate). With consolidation time, all measurements tended to increase and statistical significance (ANOVA, $p < 0.05$) was reached for most comparisons of the interior and lateral regions, but only some of the medial region.

1. 2 Chondrogenesis—Cartilage staining by alcian blue and immunohistochemistry for Col-II are shown in Fig. 4. Compared to Col-II, alcian blue stained more extensively, including not only cartilage matrix, but also osteoid, newly formed trabeculae, and tooth bud structures. Staining was often stronger at places where gap callus was approaching from both sides.

Col-II quantification is shown in Table 3. No Group A animals showed positive reaction. Group B animals were usually positive, but only in the medial region. All regions of Group C animals were positive for Col-II. Group comparison found a consistent increase with consolidation time, but only the inferior location was statistically significant (ANOVA, $P < 0.05$).

1.3 Vascular structures—Quantification of lectin binding to endothelial tissue is shown in Table 4. With the increase of consolidation time, endothelial cell density tended to increase while vessel lumen area density tended to decrease, although none reached statistical significance. Typical images showing lectin reaction in the distraction gap are shown in Fig. 5. These images suggest that the trend of increasing endothelial cell content with consolidation time was at least in part due to thickening of vessel walls.

2. The effect of periosteal injury

2. 1 Bone—The periosteum on the medial and lateral sides of the distraction site is illustrated in Fig. 6. While the medial periosteum was intact, the lateral periosteum was largely missing after distraction (Group A), but regenerated after 2 weeks of consolidation (Group C). The comparison of periosteal callus formation on medial and lateral surfaces is quantified in Table 1. Generally, the lateral side tended to grow more slowly (mineral apposition rate) and less (mineral advancement) than the medial side in Groups A and B, although only some

comparisons were statistically significant. This lateral-medial difference diminished when the lateral periosteum was restored (Group C).

Callus formation inside the distraction site is shown in Fig. 3 and Table 2. Like the periosteal callus, gap callus tended to be more advanced medially than laterally, although not all comparisons were statistically significant. This difference also diminished after two weeks of consolidation (Group C).

2.2 Chondrogenesis—Cartilage matrix, in addition to appearing first in the medial region, was usually more extensive in the medial than in the lateral and interior regions (Table 3). Unlike bone formation, this difference was still seen after two weeks of consolidation (Group C).

2.3 Vascular structures—Unlike the bone and cartilage measures, vascular structures were generally identical in the medial and lateral regions (Table 4). Compared to these regions, the interior of the distraction gap demonstrated a tendency to have lesser endothelial cell density and greater vessel lumen area density, although none of the differences was statistically significant.

3. The effect of masticatory micromovement

3.1 Bone—Masticatory micromovement of the superior and inferior locations of the distraction gap has been reported previously (23). The effect of masticatory micromovement on osteogenesis was evaluated in two ways.

First, as shown in Tables 1 and 2, the middle block, which was found to be almost immobile during mastication (27), was compared with the superior and inferior blocks, which exhibited excursions of 0.11–0.56 mm (Table 5). Most differences were statistically insignificant, but medial side values were often higher in the middle block than in the superior and inferior blocks, significantly so for gap mineral apposition rate in Group A (Table 2).

Second, Pearson correlations were performed for group B animals between gap callus measurements with regions (medial, interior and lateral) combined and the peak average micromovement of that location (Table 5). This group was chosen because growth was measured 1 week after the average peak movement measurement, which may coincide with the delay of bone biological response after a mechanical stimulation. The superior and inferior locations had similar trends, so only the inferior location is shown in Table 5. All measurements demonstrated a trend of negative correlation with mineral advancement nearly reaching significance.

3.2 Chondrogenesis—The effect of masticatory micromovement on chondrogenesis was evaluated similarly. First, the middle location was compared to the superior and inferior locations. No statistically significant difference was found (Table 3). Second, Col-II percentage measurement was examined for correlation with peak average micromovement. Again, no significant result was found (data not shown).

3.3 Vascular structures—The effect of masticatory micromovement on vascular structures was explored by correlations and no significant result was found (data not shown).

DISCUSSION

Accelerating the healing process of a mandibular osteodistraction site to shorten the overall appliance time could bring tremendous benefit to the patients. This study investigated three tissue types, bone, cartilage and the vasculature, during the early phases of healing. For each

tissue, the overall change with consolidation time was characterized, and we also examined the effect of periosteal injury and masticatory loading, both of which have been largely ignored in previous studies.

Bone

Not surprisingly, both periosteal and distraction gap callus increased with consolidation time, but their pattern differed. While periosteal callus mineral apposition rate peaked after 1 week of consolidation (Table 1, Group B), gap callus mineral apposition rate kept increasing through 2 weeks of consolidation (Group C). Comparison of these two groups of measurements clearly showed that the periosteal callus has quicker and stronger onset of formation than gap callus. What slows down the pace of periosteal mineral apposition after 1 week of consolidation? One speculation is that the periosteal callus has already approached a level adequate for functional needs. As for naturally occurring fractures, the main function of periosteal callus in DO is probably to enclose and stabilize the gap. It is reasonable that when the width of periosteal callus is mechanically adequate, callus growth should slow down. Evidence that the DO site is mechanically adequate is the fact that masticatory micromovement decreases greatly during the first week of consolidation (23).

The injury to the periosteum in this study can be attributed to two major factors. One is the trauma caused by the surgical procedures; the other is post-surgical irritation from the distractor and measuring devices. Both factors influenced the lateral periosteum more severely, hence more injury to this side. This differential injury status enabled us to evaluate the effect of periosteal injury on callus formation by comparing the lateral and medial sides. On the other hand, it is important to realize that the injury pattern observed in this study is specific to this type of surgical procedure and distractor.

Nevertheless, the comparison of the lateral and medial sides demonstrates that the contribution of periosteum to callus formation both on the surface and inside the gap was very striking. With more severe injury to the periosteum, lateral-side callus formation was significantly delayed, indicating the periosteum is a major source for new callus. This is consistent with other pig mandibular DO models (14) and is further supported by the finding that the differences between the medial and lateral sides diminished after two weeks of consolidation, when the periosteum of both sides was continuous and intact (Fig. 6).

The effect of masticatory micromovement was not as clear as that of periosteal injury, suggesting only a tendency toward inhibition. First, the minimally moved middle block (at the neutral axis) had a faster rate of mineral apposition than the more highly strained superior and inferior blocks, but this pattern was less consistent (and not significant) for mineral advancement and BV/TV. Second, a trend of negative correlation was found in Group B animals between bone measurements and average peak micromovement (Table 5), suggesting that larger movement inhibited bone formation. Although lack of statistical significance for these correlations is partly due to the small sample size, it is reasonable to conclude that masticatory micromovement was not a major influence on bone repair. Furthermore, even if the inhibitory effect was real, it was not maintained with longer consolidation, because the negative trend was not seen in Group C (data not shown). Finally, because masticatory micromovement decreases to the control level after 1 week of consolidation (23), any effect on the DO site overall healing is likely very limited.

Chondrogenesis

Chondrogenesis at a membranous bone is not usually considered a natural occurrence. In fact, another recent pig mandibular DO model did not find any cartilage formation (14). When chondrogenesis is observed in DO, mechanical explanations are often invoked (13,18,28). In

the present study, no correlation could be found between masticatory average peak micromovement and chondrogenesis, suggesting that the instability caused by mastication was not the primary cause of chondrogenesis. Instead, chondrogenesis seemed to develop in parallel with bone formation. First, the extensiveness of cartilage matrix (Collagen II linear percentage, Table 3) increased with consolidation time, significantly for the inferior block. Second, like bone formation, when compared to the lateral regions, medial region chondrogenesis was more advanced. The only difference between chondrogenesis and bone measures was that chondrogenesis in the lateral region of Group C pigs had not caught up yet to the medial region. One explanation for this difference is that chondrogenesis at the distraction gap does not depend on the periosteum as much as bone formation does. It is also likely that chondrogenesis follows and responds to the fast pace of bone formation after a short lag. In general, the data suggest that cartilage matrix formation is a normal aspect of mandibular DO repair, but that it does not precede bony development and it is not affected by the instability caused by masticatory mechanics.

Vascular structures

Endothelial cell density tended to increase with consolidation time, suggesting that angiogenesis progressed during DO healing. This finding echoes other studies that found vessel formation and upgraded expression of angiogenic factors with time (29–31). The endothelial label was due to individual positive cells in the soft tissue matrix as well as the thickening of vessel walls. During the proliferative phase of fracture healing, both fibroblasts and endothelial cells divide to form a dense matrix to replace the granulation tissue. Unlike endothelial cell density, vessel lumen density tended to decrease with consolidation time. We interpret this decrease as the natural maturation of blood vessels during the early stages of healing, a change from thin-walled sinusoidal structures to thicker-walled vessels (Fig. 6). During this process, endothelial cells may be released from the primitive vessels (32), further contributing to the increase of endothelial cell density with consolidation time.

Unlike bone formation and chondrogenesis, no difference was found between the lateral and medial regions, suggesting that vascular cells in the distraction gap do not arise mainly from the periosteum. Instead, we found that the lateral and medial regions were slightly advanced compared to the interior region (higher endothelial cell density and lower vessel lumen density). This result suggests that blood vessels from the original cortical bone may be a major source of vascular cells for the DO site.

The correlations between average peak micromovement and vascular measurements were not significant. Thus the history of peak masticatory micromovement was a negligible factor for revascularization of the DO site.

In summary, returning to the three main questions raised in the Introduction, we first found that with consolidation time, bone and cartilage formation became stronger while vascular structures became more mature. Periosteal injury had a significantly adverse effect on bone and cartilage, but not vascular development. This inhibitory effect on skeletal repair was abated with the recovery of the periosteum after 1 week of consolidation. Larger masticatory micromovement during the distraction phase tended to inhibit bone formation during early consolidation, but no effect on chondrogenesis or vascular structures was detected. Therefore, DO site interfragmentary motion caused by early soft-diet mastication poses little risk for overall DO site healing.

Acknowledgements

The authors thank Dr. Kathy Rafferty for helping with animal experiments and Ms Trisha Emry for helping with histology. This research was supported by DE 14336 from the National Institute of Dental and Craniofacial Research and by the University of Washington Orthodontic Alumni Association.

References

1. McCarthy JG, Schreiber J, Karp N, Thorne CH, Grayson BH. Lengthening the human mandible by gradual distraction. *Plast Reconstr Surg* 1992;89(1):1–8. [PubMed: 1727238]discussion 9–10
2. Ilizarov GA. Clinical application of the tension-stress effect for limb lengthening. *Clin Orthop* 1990;(250):8–26. [PubMed: 2403497]
3. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. *Clin Orthop* 1989;(239):263–285. [PubMed: 2912628]
4. al Ruhaimi KA. Comparison of different distraction rates in the mandible: an experimental investigation. *Int J Oral Maxillofac Surg* 2001;30(3):220–227. [PubMed: 11420905]
5. Farhadieh RD, Gianoutsos MP, Dickinson R, Walsh WR. Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. *Plast Reconstr Surg* 2000;105(3):889–895. [PubMed: 10724247]
6. Kessler PA, Merten HA, Neukam FW, Wiltfang J. The effects of magnitude and frequency of distraction forces on tissue regeneration in distraction osteogenesis of the mandible. *Plast Reconstr Surg* 2002;109(1):171–180. [PubMed: 11786809]
7. Paccione MF, Mehrara BJ, Warren SM, Greenwald JA, Spector JA, Luchs JS, et al. Rat mandibular distraction osteogenesis: latency, rate, and rhythm determine the adaptive response. *J Craniofac Surg* 2001;12(2):175–182. [PubMed: 11314629]
8. Cheung LK, Zheng LW, Ma L. Effect of distraction rates on expression of bone morphogenetic proteins in rabbit mandibular distraction osteogenesis. *J Craniomaxillofac Surg* 2006;34(5):263–269. [PubMed: 16781161]
9. King GJ, Liu ZJ, Wang LL, Chiu IY, Whelan MF, Huang GJ. Effect of distraction rate and consolidation period on bone density following mandibular osteodistraction in rats. *Arch Oral Biol* 2003;48(4):299–308. [PubMed: 12663075]
10. Troulis MJ, Glowacki J, Perrott DH, Kaban LB. Effects of latency and rate on bone formation in a porcine mandibular distraction model. *J Oral Maxillofac Surg* 2000;58:507–513. [PubMed: 10800906]
11. Cope JB, Samchukov ML. Regenerate bone formation and remodeling during mandibular osteodistraction. *Angle Orthod* 2000;70:99–111. [PubMed: 10832997]
12. Djasim UM, Wolvius EB, van Neck JW, Weinans H, van der Wal KG. Recommendations for optimal distraction protocols for various animal models on the basis of a systematic review of the literature. *Int J Oral Maxillofac Surg* 2007;36(10):877–883. [PubMed: 17616340]
13. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. *Clin Orthop* 1989;(238):249–281. [PubMed: 2910611]
14. Glowacki J, Shusterman EM, Troulis M, Holmes R, Perrott D, Kaban LB. Distraction osteogenesis of the porcine mandible: histomorphometric evaluation of bone. *Plast Reconstr Surg* 2004;113(2):566–573. [PubMed: 14758219]
15. Malizos KN, Papatheodorou LK. The healing potential of the periosteum molecular aspects. *Injury* 2005;36 Suppl 3:S13–19. [PubMed: 16188544]
16. Hasse AR, Porksen M, Zimmermann CE. Bilateral mandibular distraction in adult dogs with an epiperiosteal distractor. *Br J Oral Maxillofac Surg* 2005;43(2):105–112. [PubMed: 15749209]
17. Aronson J, Harrison B, Boyd CM, Cannon DJ, Lubansky HJ. Mechanical induction of osteogenesis: the importance of pin rigidity. *J Pediatr Orthop* 1988;8(4):396–401. [PubMed: 3260601]
18. Aronson J. Experimental and clinical experience with distraction osteogenesis. *Cleft Pal Craniofac J* 1994;31:473–481.

19. Lienau J, Schell H, Duda GN, Seebeck P, Muchow S, Bail HJ. Initial vascularization and tissue differentiation are influenced by fixation stability. *J Orthop Res* 2005;23(3):639–645. [PubMed: 15885486]
20. Kassis B, Glorion C, Tabib W, Blanchard O, Pouliquen JC. Callus response to micromovement after elongation in the rabbit. *J Pediatr Orthop* 1996;16(4):480–483. [PubMed: 8784701]
21. Kenwright J, Goodship AE. Controlled mechanical stimulation in the treatment of tibial fractures. *Clin Orthop Relat Res* 1989;(241):36–47. [PubMed: 2924478]
22. Buckwalter JA. Effects of early motion on healing of musculoskeletal tissues. *Hand Clin* 1996;12(1):13–24. [PubMed: 8655614]
23. Sun Z, Rafferty KL, Egbert MA, Herring SW. Masticatory mechanics of a mandibular distraction osteogenesis site: interfragmentary micromovement. *Bone* 2007;41(2):188–196. [PubMed: 17532283]
24. Henry CB, DeFouw DO. Differential lectin binding to microvascular endothelial glycoconjugates during normal angiogenesis in the chick chorioallantoic membrane. *Microvasc Res* 1995;49(2):201–211. [PubMed: 7541505]
25. Minamikawa T, Miyake T, Takamatsu T, Fujita S. A new method of lectin histochemistry for the study of brain angiogenesis. *Lectin angiography. Histochemistry* 1987;87(4):317–320. [PubMed: 3319974]
26. Kassab GS, Fung YC. Topology and dimensions of pig coronary capillary network. *Am J Physiol* 1994;267(1 Pt 2):H319–325. [PubMed: 8048597]
27. Sun Z, Rafferty KL, Egbert MA, Herring SW. Mandibular mechanics after osteotomy and distraction appliance placement I: Postoperative mobility of the osteotomy site. *J Oral Maxillofac Surg* 2006;64(4):610–619. [PubMed: 16546640]
28. Meyer U, Meyer T, Wiesmann HP, Kruse-Losler B, Vollmer D, Stratmann U, et al. Mechanical tension in distraction osteogenesis regulates chondrocytic differentiation. *Int J Oral Maxillofac Surg* 2001;30(6):522–530. [PubMed: 11829235]
29. Hu J, Zou S, Li J, Chen Y, Wang D, Gao Z. Temporospatial expression of vascular endothelial growth factor and basic fibroblast growth factor during mandibular distraction osteogenesis. *J Craniomaxillofac Surg* 2003;31(4):238–243. [PubMed: 12914709]
30. Li G, Simpson AH, Kenwright J, Triffitt JT. Effect of lengthening rate on angiogenesis during distraction osteogenesis. *J Orthop Res* 1999;17(3):362–367. [PubMed: 10376724]
31. Matsuyama J, Ohnishi I, Kageyama T, Oshida H, Suwabe T, Nakamura K. Osteogenesis and angiogenesis in regenerating bone during transverse distraction: quantitative evaluation using a canine model. *Clin Orthop Relat Res* 2005;(433):243–250. [PubMed: 15805964]
32. Bauer SM, Bauer RJ, Velazquez OC. Angiogenesis, vasculogenesis, and induction of healing in chronic wounds. *Vasc Endovascular Surg* 2005;39(4):293–306. [PubMed: 16079938]

Fig. 1.

A) Specimen collection. Two blocks were from the superior (above the distractor) and two from the inferior (below the distractor) locations, respectively. The blocks closest to the hardware (hatched boxes) were processed with decalcification. The most superior and inferior blocks (filled boxes) were processed without decalcification (filled boxes). One block (clear box) was collected from the middle location (underneath the distractor) and processed either with or without decalcification (see text for details). All blocks were sectioned in a plane parallel to the distractor rod. B) Diagram of a typical section. Newly formed callus spicules were wavy and thin. The solid-line boxes indicate surface callus. The broken-line boxes indicate gap callus, M, medial region; I, interior region and L, lateral region.

Fig. 2.

A) Mineralization measurement in undecalcified sections illustrated by periosteal callus. Mineral apposition rate was the average distance between the fronts of calcein (green label, line b) and alizarin complexone (red label, line a) divided by 3 days. Mineral advancement was the average distance between the old bone front (line c) and the new callus front (line a). B) Periosteal callus bone volume fraction (BV/TV) measurement in decalcified sections. A square 19×25 test grid was superimposed on the image. BV/TV was defined as the number of intersections hitting the callus bone divided by the number of intersections hitting the entire callus area (enclosed by broken white lines). C) Linear percentage of Col-II (+) matrix measurement shown on a decalcified section reacted for anti-Col II. The bone margins of the DO gap on both sides are indicated by solid lines (a). The portions of the margins in relation to Col-II (+) (brown color, arrows) are indicated by broken lines (b). Col-II linear percentage was defined as the total length of b divided by the total length of a. D) Assessment of vascular structures illustrated on an image of the interior gap site. Lectin immunohistochemistry was used to reveal endothelial cells (brown). The site of interest was defined as the distraction gap soft tissue (inside of the broken lines). Color thresholding was performed to dichotomize the image (not shown) for quantification of endothelial cell density (thresholded area divided by the total area of site of interest) and blood vessel lumen area density (total area of vessel lumens divided by the total area of the site of interest). Calibration bars, 500 μ m.

Fig. 3.

Distraction gap callus formation. The left space in each image is the distraction gap. A) Lateral and B) medial regions of a Group A distraction site. Note callus was mainly forming in the medial region and arose from the periosteal callus. C) Lateral and D) medial regions of a Group C distraction site. Note callus was forming in both medial and lateral regions and was from both periosteal callus and the old cortex. E) and F), Interior regions from different animals. E shows callus formation originating from old trabecular bone (arrowheads). F shows callus formation originating from periosteal callus (arrows) and growing towards the old bone surface (*). Calibration bars, 500 μ m.

Fig. 4. Chondrogenesis in the interior region of the distraction gap of Group C animals. Cartilage matrix was revealed by alcian blue staining (blue) and Collagen II immunohistochemistry (brown). Broken lines indicate bone fronts.

Fig. 5.

Vascular structures inside the distraction gap. Positive (lectin-stained) cells were thresholded and pseudocolored (orange). Broken lines indicate the borders between distraction gap soft tissue and bone matrix. Differences in background color are due to variation of white balancing between sections. A), Group A (no consolidation), B) Group C (2 weeks of consolidation). With consolidation time, blood vessel walls thickened while lumens shrank. Calibration bars, 100 μm .

Fig. 6.

Periosteal condition and new bone formation. At the end of distraction (A and B), the lateral periosteum was not continuous (arrow heads) but the medial periosteum was intact (arrows). New bone formation was stronger at the medial surface. After two weeks of consolidation (C and D), the lateral periosteum (arrows) was repaired and new bone formation was catching up with the medial side. Calibration bars, 1000 μ m

Table 1
Periosteal callus measurements (Mean±SD)

Mineral apposition rate (mm/day)	Superior block		Inferior block		Middle block	
	Medial	Lateral	Medial	Lateral	Medial	Lateral
Group A ^a (nd=4/4/4)	0.08±0.04	0.04±0.03	0.11±0.04	0.04±0.04	0.12±0.04	0.05±0.05
Group B ^b (n=5/5/4)	0.17±0.03 [*]	0.09±0.02	0.13±0.06	0.07±0.03	0.09±0.08	0.06±0.05
Group C ^c (n=5/5/0)	0.03±0.02	0.02±0.02	0.06±0.01	0.06±0.06	ND	ND
Group ANOVA: p	0.002	0.001	0.132	0.783		
Mineral advancement (mm)						
Group A (n=4/4/4)	0.45±0.18	0.12±0.08	0.54±0.17 [*]	0.11±0.08	0.84±0.06 [*]	0.05±0.02
Group B (n=5/5/4)	1.32±0.78	1.01±0.64	1.02±0.28 [*]	0.62±0.24	1.23±0.37 [*]	0.26±0.19
Group C (n=5/5/0):	0.83±0.34	0.89±0.64	1.36±0.47	0.82±0.43	ND	ND
Group ANOVA: p	0.082	0.073	0.014	0.014		
Callus BV/TV (%)						
Group A (n=4/4/0):	35.8±6.5	33.6±9.5	40.7±4.6 [*]	11.9±13.9	ND	ND
Group B (n=6/6/3)	42.5±12.6	37.7±10.1	52.9±8.2	45.8±13.6	59.4±6.4 ^{*ζ}	42.5±10.8
Group C (n=5/5/5)	60.3±8.8	57.9±13.5	66.8±11.7	57.8±12.5	72.4±4.2	59.4±21.3
Group ANOVA: p	0.008	0.012	0.003	<0.001		

^a, Group A had distraction only, no consolidation and 7-day post-op survival period
^b, Group B had distraction, about 1-week consolidation and 14-day post-op survival period
^c, Group C had distraction, about 2-week consolidation and 21-day post-op survival period
^d, Number of samples with available data at superior/inferior/middle blocks
^{*}, The medial surface is significantly greater (paired t-test, p<0.05) than the lateral surface
^ζ, The middle block is significantly greater than the superior and inferior blocks (one-way ANOVA, p<0.05)

Table 2
Distraction gap callus formation measurements (Mean±SD)

Mineral apposition rate (mm/day)	Superior block			Inferior block			Middle block		
	Medial	Interior	Lateral	Medial	Interior	Lateral	Medial	Interior	Lateral
Group A (n ^a =4/4/4);	0.02±0.006 [*]	0.01±0.005	0.005±0.002	0.02±0.01 [*]	0.004±0.002	0.004±0.001	0.06±0.02 ^{*§}	0.02±0.03	0.02±0.03
Group B (n=5/5/3);	0.04±0.04	0.03±0.03	0.03±0.03	0.03±0.03	0.01±0.01	0.02±0.01	0.02±0.03	0.01±0.02	0.01±0.01
Group C (n=5/5/0);	0.04±0.04	0.06±0.03	0.04±0.02	0.05±0.04	0.05±0.03	0.03±0.02	ND	ND	ND
Group ANOVA: p	0.627	0.041	0.093	0.354	0.006	0.045			
Mineral advancement (mm)									
Group A (n=4/4/4);	0.34±0.23	0.05±0.04	0.05±0.05	0.28±0.25 [*]	0.01±0.003	0.03±0.01	0.42±0.17 ^{**}	0.11±0.08	0.05±0.06
Group B (n=5/5/3);	1.57±0.92	0.79±0.45	0.79±0.65	1.07±0.50	0.49±0.56	0.44±0.49	0.33±0.08 [*]	0.14±0.13	0.05±0.01
Group C (n=5/5/0);	1.71±0.94	1.02±0.66	1.08±0.24	1.71±0.71	1.31±0.54	0.76±0.61	ND	ND	ND
Group ANOVA: p	0.050	0.032	0.011	0.008	0.005	0.112			
Callus BV/TV (%)									
Group A (n=4/4/0);	22.3±8.1 ^{**}	7.6±4.0	1.8±2.0	15.8±6.6 [*]	5.4±5.1	1.3±1.3	ND	ND	ND
Group B (n=5/5/3);	29.3±7.4 ^{**}	10.8±7.4	9.0±5.2	28.4±13.5	19.6±17.0	16.2±12.2	26.9±12.1	34.0±10.4	27.4±16.2
Group C (n=5/5/5);	33.2±15.9	31.9±12.9	29.0±9.1	51.3±10.4	51.6±8.6	40.7±12.2	42.1±8.9	46.1±9.4	42.5±13.9
Group ANOVA: p	0.389	0.004	<0.001	0.001	<0.001	<0.001			

^a, Number of samples with available data at superior/inferior/middle blocks;

^{*}, The medial region was significantly greater than the lateral and interior regions, ANOVA, p<0.05,

^{**}, p<0.01;

[§]The middle block was significantly greater than the superior and inferior blocks, ANOVA, P<0.05

Table 3

Col-II reaction linear percentage

Animal ID	Superior block (%)			Inferior block (%)			Middle block (%)		
	Medial	Interior	Lateral	Medial	Interior	Lateral	Medial	Interior	Lateral
Group A (n ^a =4/4/0)	0	0	0	0	0	0	ND	ND	ND
Group B (n=6/6/1)	16.4±20.4 [*]	0	0	7.7±13.0	0	0	32.0	7.2	9.7
Group C (n=5/5/5)	20.0±16.1 [*]	2.3±5.1	2.6±5.8	50.6±19.6 [*]	7.6±7.9	20.6±21.1	46.4±23.4 [*]	9.5±12.2	12.4±11.4
Group ANOVA: p	0.191	0.391	0.395	<0.001	0.03	0.03	-	-	-

^a , Number of samples with available data at superior/inferior/middle blocks;

^{*} , The medial region was significantly greater than the interior and lateral regions, ANOVA, p<0.05.
ND, no data.

Table 4

Distraction gap vascular structures (Mean±SD)

Endothelial cell density (%)	Superior block			Inferior block		
	Medial	Interior	Lateral	Medial	Interior	Lateral
Group A (n ^a =4/4)	2.7±2.1	1.8±0.7	3.1±1.7	3.1±2.2	1.8±1.6	2.2±2.5
Group B (n=6/6)	2.7±1.2	2.4±1.6	3.1±1.7	2.6±1.8	2.2±0.9	3.4±1.6
Group C (n=5/5)	4.5±0.4	4.0±1.2	3.7±1.6	3.6±1.1	5.2±4.9	3.9±3.3
Group ANOVA: p	0.084	0.058	0.809	0.641	0.195	0.60
Blood vessel lumen area density (%)						
Group A (n=4/4)	3.0±1.0	6.0±0.5	3.6±2.0	4.4±3.8	6.8±2.5	6.4±1.3
Group B (n=6/6)	4.9±1.9	4.1±1.4	4.4±2.1	3.9±1.5	4.9±2.6	4.8±3.8
Group C (n=5/5)	3.0±1.0	4.4±2.1	2.6±0.6	2.3±0.8	4.7±2.1	3.7±1.1
Group ANOVA: p	0.089	0.178	0.263	0.336	0.402	0.338

^a, Number of samples with available data at superior/inferior blocks.

Table 5
The effect of masticatory micromovement on bone formation (Group B, inferior location)

Animal ID	Peak Average Micromovement (mm)	Bone		
		MAR (mm/day)	Mineral advancement (mm)	BV/TV (%)
B1	0.39	0.021	0.55	6.1
B2	0.56	0.008	0.23	12.8
B4	0.44	0.027	0.36	31.7
B7	0.16	0.014	0.70	32.7
B8	0.11	0.064	1.51	23.4
Pearson correlation: r (p)		-0.63 (0.250)	-0.87 (0.058)	-0.46 (0.433)