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The Effect of Pulsed Ultrasound on Mandibular Distraction

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Abstract—This study evaluated the effect of pulsed ultrasound on tissue repair and bone growth during mandibular osteodistraction. Twenty-one rabbits were divided into three groups of 7. The distraction started 72 h after surgically severing both sides of the mandible and proceeded at a rate of 1.5 mm/12 h for 5 days. Group 1 received pulsed ultrasound (nominally 200 μs pulse of 1.5 MHz at a 1.1 kHz pulse repetition frequency, 30 mW/cm^2) for 20 min on both sides of the mandible every other day (alternating sides). Group 2 received the same pulsed ultrasound treatment on one side of the mandible every day for 20 min. Group 3 did not receive any ultrasound treatment. Bone formation at the distraction site was assessed by photodensitometry on head radiographs, a vibratory coherence test across the distraction site, a postmortem three-point bending mechanical stiffness test, and a postmortem histological examination. Statistical analyses performed using analysis of variance revealed that pulsed ultrasound enhanced bone formation at the distraction site with a high level of significance when assessed by the increase in new bone photodensity ($p=0.001$), vibratory coherence ($p=0.001$), mechanical stiffness ($p=0.003$), and qualitative histological studies, especially when the pulsed ultrasound treatment was directly applied daily. © *2002 Biomedical Engineering Society.* [DOI: 10.1114/1.1529196]

Keywords—Bone healing, Bone formation, Distraction osteogenesis, Therapeutic pulsed ultrasound.

INTRODUCTION

Therapeutic pulsed ultrasound (US) has been used to enhance bone healing after fracture in a variety of human and animal models (e.g., Refs. 13, 17, and 21). Recently, the technique has been applied successfully to enhance growth and healing after distraction of the tibia in a rabbit model[24] and after distraction of the callus in the metatarsus in a sheep model.[18] The present article reports on a study that addresses the question of whether it also would enhance bone formation during mandibular distraction osteogenesis.

Distraction osteogenesis has been employed successfully to gain increased bone and soft tissue mass in patients with a variety of craniofacial deformities.[19,20] Intraoral tooth-borne distraction devices have been used to facilitate complex multiplanar distraction.[22] However, the final result of bone lengthening in the mandible may be modified because masticatory muscle forces may lead to bending of the newly formed bony callus.[2] Therefore, several groups have studied techniques to enhance bone healing during distraction osteogenesis (e.g., insulin-like growth factor[25] and electrical stimulation).[12] However, these chemical and electrical techniques require injections with growth factors, or are invasive, which may make them unsuitable for clinical application. Since pulsed US can be applied at the skin surface noninvasively it may be preferable in a wide range of clinical applications.

The objective of this study was to evaluate the efficacy of pulsed US to promote bone healing and to allow an increased distraction rate in mandibular osteodistraction in a rabbit model with healthy end results. An increased rate would be preferable to the patient. Relative to the diaphyseal bones used in other studies, such as the tibia, the mandible has a complex geometry and evaluation of the healing process and the end result may prove more difficult. Consequently, four different independent methods (radiographic, vibratory, mechanical, and histological) were used in this study for comparative evaluation of the elongated bone health of rabbits subjected to different pulsed US treatment protocols.

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EXPERIMENTAL DESIGN AND METHODS

Animal Model

Twenty-one New Zealand white, male, skeletally mature rabbits weighing 3–3.6 kg were divided into three groups of seven each, groups 1, 2, and 3. Each group received different levels of pulsed US treatment as described later. The rabbit model has been used in many previous studies that deal with distraction osteogenesis of the mandible (e.g., Refs. 11, 12, 16, 25, and 26) and in studies applying pulsed US to enhance bone fracture healing.[3,7] However, to the best of the authors' knowledge there are no such studies (with rabbit or other models) using pulsed US therapy to promote healing after mandibular osteodistraction. The experimental protocol was approved by the Animal Care Committee at the University of Illinois at Chicago (No. 1999-076).

Distraction Rate

Distraction was started 72 h after surgery by opening the screws 1.5 mm each 12 h for 5 days. Stewart *et al.*[26] showed that there were no statistical differences in the mechanical stiffness testing or the bone density between two groups of rabbits that underwent distraction at rates of 0.5 and 1.5 mm per 12 h. However, there was fibrous union in the middle of the distraction zone in the rapidly distracted group and it was concluded that the slower distraction rate produced better end results. In the present study, it was decided to use the rapid distraction rate, which would be beneficial to the patient if pulsed US can produce quality of bone comparable to that created by a distraction rate of 1 mm per day, or normal bone.

Choice of Distraction Device

The distraction devices used in this study are primarily used in distraction of human mandibles.[5,22] The advantage of using such devices is that they provide anteriorly activated distraction screws that enable the patient, the clinician, or the researcher to activate the device from the anterior of the distraction site, which provides accessibility for performing the distraction with comfort. The devices were reduced in size and their geometry was slightly modified to fit the rabbits' mandibles in this study.

Surgery

The surgery for mandibular distraction osteogenesis was performed on all animals using aseptic techniques. Animals were anesthetized (xylazine, 5 mg/kg; ketamine, 44 mg/kg IM; and inhalation of 1.5%–3% Isoflurane gas to maintain anesthesia); then, the operative area was shaved and disinfected with aqueous Betadine (0.5 ml/kg). Both sides of the mandible were exposed through a submandibular incision, and an osteotomy was made on the buccal (outer side) through the anterior part of the mandible (just anterior to the first molar) with a water-cooled long tapered fissure bur (Brasseler, Savannah, GA).

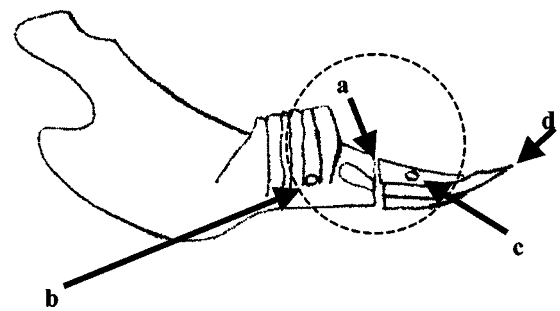

FIGURE 1. Diagram showing: (a) the osteotomy site; (b) posterior hole for fixation of the distraction device and mounting of the accelerometer; (c) anterior hole for fixation of the distraction device; and (d) impulse hammer contact point. Dashed circle denotes approximate region covered by pulsed US transducer that was gently pressed against the shaved skin surface using ultrasound coupling gel and a custom-made fabric sling that encircled the rabbit's jaw and nose. In the diagram, posterior is to the left, anterior is to the right, and lateral is normal to the page.

The anterior segment of the mandible was chosen as the distraction site for the following reasons: (1) to allow the cut to be made through the anterior part of the rabbit mandible; (2) to facilitate placement of the distraction device; and (3) to facilitate the application of the pulsed US treatment to either or both sides of the mandible. Four holes were drilled, two on each side of the corticotomy; pins were placed in the holes to stabilize the external distractor, which was positioned under the inferior border of the mandible. Figure 1 shows a diagram of the surgical site and the holes for fixation screws to hold the distraction device in place. During surgery, a 5–8 French nasoesophageal feeding tube (Medovations Inc., Wauwatosa, WI) was inserted and sutured to the rabbit's face for postoperative feeding. An E-collar (Saf-T-Shield, EJAY International Inc., Glendora, CA), was inserted around the rabbits' heads to prevent them from dislodging the nasoesophageal tubes or the distraction devices.

Postoperative care included injecting the animals with 0.02 mg/kg Buprenorphine (Reckitt and Colman Products, Hull, England HU8 7DS) subcutaneously to alleviate postoperative pain. This was repeated twice per day for 2 days. Also, a prophylactic antibiotic was given to the rabbits, Enrofloxacin 5 mg/kg (Bayer Shawnee, Mission, KS) subcutaneous twice per day. Postoperative feeding was performed using the nasoesophageal tube until the rabbits were able to eat independently (which usually occurred after a few days); then the nasoesophageal tube was removed. The animals were monitored to assess hydration and food intake. Animal weights were checked daily and compared to the presurgical values. If an animal's weight decreased by 20% of their initial body weight, the nasogastric tube was reinserted for feeding. (This occurred in 2 of the 21 rabbits in the study.)

All rabbits were treated with similar distraction devices and the surgery was performed using the same technique and by the same surgeon. All animals were sacrificed 5 weeks postsurgery because previous mandibular distraction osteogenesis studies found that the major significant differences in bone formation occurred during the first 3–4 weeks.[11,25]

Pulsed Ultrasound Treatment

Pulsed US was applied to rabbit groups 1 and 2 while under sedation with Domitor 0.25 mg/kg IM (Pfizer Inc., Espoo, Finland). Group 1 received pulsed US treatment to both sides of the mandible every other day alternating sides each day for 20 min. Group 2 received pulsed US on the left side of the mandible daily for 20 min, but was not treated on the right side. Group 3 was used as the control group and did not receive any pulsed US treatment. Three groups were chosen in this way in order to compare both dose and duration, as the other side of the mandible would receive a lower, but non-negligible, exposure level relative to the closer side. So, in terms of duration of exposure, both right and left sides of the mandible of group Nos. 1 and 2 were the same, with group No. 3 being the control. But in terms of dose, the left side of group No. 2 received the higher (direct) dose level every day. The left and right sides of group No. 1 received the higher (direct) and lower (indirect) dose levels on alternating days. The right side of group No. 2 received the lower (indirect) dose level every day.

Pulsed US was applied for 20 min per day during and for 4 weeks after distraction using a commercially available instrument (Exogen Inc., West Caldwell, NJ). The unit has a 2.5 cm diameter transducer nominally generating a 200 μm duration burst of 1.5 MHz ultrasound at a pulse repetition frequency of 1.12 kHz delivering 30 mW/cm^2 temporally and spatially averaged incident intensity. The transducer was pressed securely against the outer surface of the mandible of each rabbit with Velcro straps using a special sling tailored for the size of the rabbit's head. Ultrasound gel (Exogen Inc., West Caldwell, NJ) was used to couple the transducer and the shaved skin surface. The location of the transducer overlying the distraction zone is indicated in Fig. 1.

Ultrasound power was determined from the measurement of radiation force using a technique as described by Rooney.[23] Briefly, the radiation force was measured by mounting the ultrasound transducer on a holder that allowed the ultrasound transducer to be immersed in degassed water in a container that had an acoustic absorbing material (Wallgone) at its bottom. The ultrasound transducer was fully immersed in the water and was in close proximity to (within 2–4 mm) the absorber. The weight of the whole assembly was measured using a digital analytical balance with 0.1 mg precision. The reading was recorded after the digital balance stabilized. The change in the weight of the container was observed after turning the ultrasound device on, and after the balance reading was again stabilized. The difference between the balance reading before and after turning the ultrasound device on was measured three times and the results averaged. Then, the radiation force F_R was calculated as

$$F_R = W_A / c, \tag{1}$$

where W_A is the total acoustic power emitted by the ultrasound source and c is the ultrasound propagation speed in water. Using a value for c (1491 m/s) corresponding to room temperature (21 °C), the earlier relation gives a conversion factor of 68.5 mg/W. Total acoustic power measurements were made before and after each animal exposure.

After the application of pulsed US to groups 1 and 2, the rabbits were awakened using Antisedan at a dose of 1 mg/kg IM (Pfizer, Espoo, Finland). In hindsight, group 3 should also have been given the same sedation and antisedation dosages to keep the pulsed US treatment as the only variable. However, to the best of the authors' knowledge, there has not been a report correlating the use of Domitor (sedation) and Antisedan (anti-sedation) with bone formation. In all animals the upper incisors were ground down as needed after distraction to alleviate ulceration of the lower gingiva, as the animals developed crossbite (reverse bite) of the incisors at the end of the distraction.

Radiographic In Vivo Assessment

Predistraction and weekly postdistraction lateral head radiographs were taken with an aluminum step-wedge attached to the radiographic film holder, so as to project on the same film as the part of the mandible being examined. The radiographs were taken with x-ray machine settings of 65 kV peak, 300 mA, and a 1/60 s exposure. The animals were under short period sedation during radiographic examination using Domitor (0.25 mg/kg IM; Pfizer). The step-wedge ranged in thickness from 1 to 9 mm in steps of 1 mm and was used to standardize the photodensity of each radiograph in order to evaluate the quality of the newly formed bone using a photodensitometer (Macbeth TD501, Macbeth Division of Kollmorgen Corp.). Bone density was calculated according to Ref. 4 using the following formula:

$$D=(\ln[\mathrm{DR}]^2-a)/b. \quad (2)$$

Here, D is the photodensity of the bone area being studied in mm aluminum equivalent, DR is the densitometer reading, ln denotes the natural logarithm, and "a" and "b" are the least squares regression coefficients for each radiograph obtained from the following equation:

$$\ln[\mathrm{DR}]^2=a+bh, \quad (3)$$

where h is the height of the step-wedge level used and DR is the densitometer reading for that particular aluminum height.

The percentage of the photodensity of the newly formed bone was compared as a percentage to the normal bone next to the distraction site. In lateral head radiographs it was difficult to distinguish between the right and the left sides during distraction; so, another vertical radiograph was taken for each mandible after dissection to measure bone photodensity for both sides of the mandible. In radiographs taken for the rabbits during distraction (lateral head only), normal bone photodensity *in vivo* was higher than after dissection by 5%. So, photodensities for normal and new bone formed were corrected by this factor to calculate the actual bone photodensity during the 4 weeks of distraction.

Note, the technique employed in this study is one of several radiographic techniques that can be used to estimate bone density, along with other potentially more accurate (and expensive) approaches based on DXA, QCT, and pQCT technology.[8] Since density estimates alone, no matter how accurate, do not exactly correlate to the strength or maturation of bone, additional measurement modalities were employed in this study.

Vibratory Coherence Assessment

Evaluation of bone healing was also assessed via a novel vibratory coherence technique that is described in more detail in a prior publication.[6] Due to the complex geometry of the mandible, it was found that other more common vibratory techniques, such as resonant frequency measurements, were not useful. A painless *in vivo* mechanical impulse excitation was delivered to the rabbit lower incisor at its tip (proximal to the distraction site) using an instrumented hammer (PCB No. GK291c, Depew, NY) that generates an electric signal proportional to the impulse force (see Fig. 1). A response measurement was made using an accelerometer (PCB No. 352a10) rigidly adhered via a custom-made connector to a screw (3 mm diam, 15 mm length) driven 4 mm through the posterior end of the rabbit's mandible (posterior to the distraction site by 20 mm). The accelerometer signal and the force sensor signal from the hammer tip were recorded and digitized using a two-channel spectrum fast Fourier transform analyzer (Agilent No. 35670A). For each measurement, eight impulse excitation and accelerometer response time records were Fourier transformed and averaged to improve the signal-to-noise ratio. The coherence γ_{xy}^2 between the hammer force sensor and accelerometer was calculated. The coherence gives a measure of the linear deterministic relationship between the input and output; in other words, if the signal-to-noise ratio is poor or the system has significant nonlinearity, the coherence will be poor, approaching zero. A value of one denotes perfect coherence; the output is entirely determined by the input and the input-output relationship is linear. It is hypothesized that healthy bone, without fracture or discontinuity, will produce a higher coherence between input/output measurements located at different points on the bone. Further discussion of this technique can be found elsewhere.[6] This test was performed during the surgery, before performing the osteotomy as a base line for each rabbit, and post distraction. The area under the coherence curve for each rabbit was integrated over the frequency range from 0 to 800 Hz to obtain one representative number for each animal. Note, while the coherence test is painless, to ensure animal cooperation and to minimize stress and discomfort it was performed when the rabbits were anesthetized for surgery, but before surgical sectioning of the mandible and immediately after they were sacrificed.

Postmortem Preparation for Mechanical Stiffness Measurement and Histological Examination

Four weeks after completion of distraction, all rabbits were sacrificed by intravenous injection of 1 ml/kg sodium pentobarbitone (Vortech Pharmaceuticals Inc., Dearborn, MI). For each rabbit, the mandible was dissected and split into two halves. Half of each mandible was stored in phosphate buffered saline at 4 °C for 2–3 weeks until mechanical testing was performed. The other half was fixed in 10% buffered formalin for 2 weeks and then decalcified using a solution containing equal volumes of 50% formic acid and 20% sodium citrate. The distraction site and parts of the original bone, anterior and posterior to the distraction site, were subsequently dissected and embedded in paraffin. Thick sections (6 μm) were cut and stained with hematoxylin and eosin for light microscopy. (Note, for group 2 rabbits, both left and right mandibles were needed for mechanical stiffness measurements and histology. So, for the seven rabbits of group 2 that underwent photodensity and vibratory coherence measurements, both left and right mandible halves were prepared for stiffness measurements. Then, an additional five rabbits that underwent the same protocol as the group 2 animals had both their mandible halves, left and right, prepared for qualitative histology studies using light microscopy.)

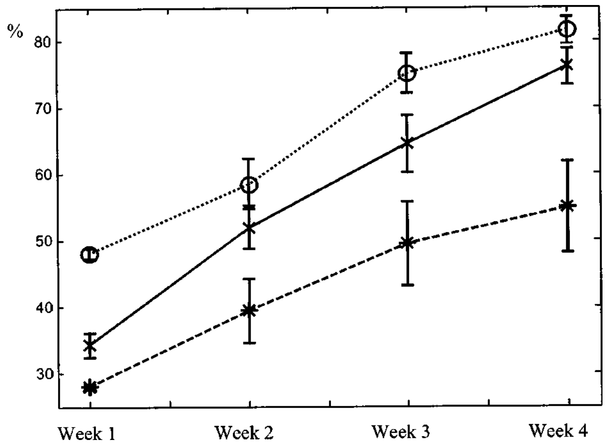

FIGURE 2. Photodensity percentage of newly formed bone relative to normal bone next to distraction site at the end of each week after distraction based on lateral head radiograph. Key: ×——× group 1—pulsed US alternating sides, ○– – –○ group 2—pulsed US same side,*——*-group 3—no pulse US. Error bars denote +/−1 standard error.

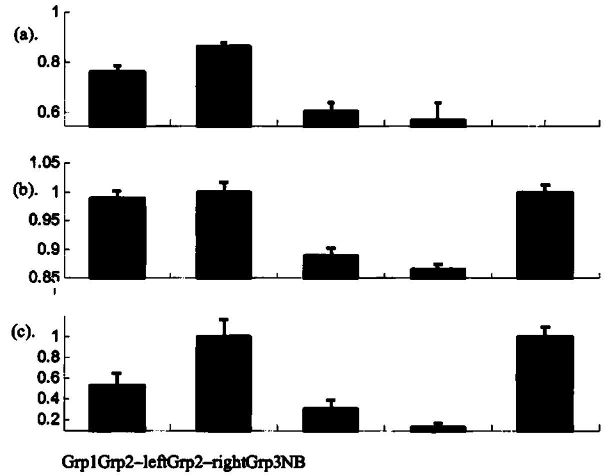

FIGURE 3. Normalized (a) photodensity, (b) coherence, and (c) mechanical stiffness at 4 weeks after distraction. Photodensity is normalized relative to normal bone next to the distraction site. For the coherence and mechanical stiffness measurements an additional seven rabbits, which did not undergo distraction were tested (NB—normal bone) and used to normalize data. Error bars denote+1 standard error.

Postmortem Mechanical Stiffness Assessment

Mechanical three point bending of the distracted mandibular half was performed using an Instron No. 5500 servohydraulic materials testing machine (Instron, Canton, MA). The samples were tested in a simply supported configuration. The samples were loaded on the lateral aspects of the mandibles.[6] The displacement rate was 1 mm/s. The stiffness of the half mandibular structure was calculated as the slope of the initial linear segment on the load deflection curve. (Further extrapolations based on the measured data, such as a calculation of elastic moduli, were not attempted given the complex geometry of the mandible.)

The three point bending technique has been used by many authors to test bone fracture healing and mechanical properties of newly formed bone at the distraction site,[26] and to test normal bone fracture healing.[1] Due to the presence of fibrous tissue in most of the specimens in group 3, it was not always possible to obtain and compare peak load to fracture in all specimens; consequently, only the stiffness measurement was used to compare the relative degree of bone healing in all groups.

In order to obtain samples of normal bone for mechanical stiffness measurements, seven additional rabbits were obtained from another study that did not affect bone metabolism, bone mineral content, or have any head and neck surgical interference. The mandibles were dissected and stored in phosphate buffered saline at 4 °C exactly like the experimental specimens until mechanical measurements were made.

RESULTS

Measurement of Pulsed US Power

Before the animal experiments began, the total pulsed US power was measured during three successive simulated applications where the instrument was turned on for a period of 20 min. The spatially averaged, temporally averaged intensity (I_{SATA}) was computed by dividing the total power by the transducer area. It was found that during the first application, I_{SATA} was slightly higher than the range prescribed by the manufacturer (30 ± 5 mW/cm^2). During the second application, I_{SATA} was within the range prescribed by the manufacturer. The I_{SATA} in the third application was lower than that specified by the manufacturer. Thus, each instrument was used for only two applications before the batteries were recharged according to the manufacturer's guidelines. The ultrasound power was then measured prior to and after each animal exposure and showed less than 5% change in output.

Radiographic Assessment

Figure 2 shows the mean and standard errors of the percentage of photodensity of the newly formed bone at the distraction site normalized to the normal bone in all groups for four weeks after distraction using lateral head radiographs. The left and right sides of group 2 were only differentiated after 4 weeks postmortem as discussed in the previous section; separated results for the left and right side photodensity measurements are presented in Fig. 3(a) and in Table 1. Referring to Fig. 2, it appears that the pulsed US treatment has substantially increased bone photodensity, with a greater increase in group 2, which received treatment to the same side every

TABLE 1. Photodensity percentage, coherence, and mechanical stiffness measurements at 4 weeks after distraction. Photodensity percentage is the percentage value relative to normal bone next to the distraction site. Coherence measurements are normalized to predistraction values. For the coherence and mechanical stiffness measurements an additional seven rabbits that did not undergo distraction were tested (NB—normal bone). Key: Mean +/−1 standard error.

Groups	1	2-Left	2-Right	3	NB
Photodensity %	75.9+/−2.6	86.0+/−1.8	60.6+/−3.5	57.0+/−7.0	···
Coherence	0.89+/−0.01	0.90+/−0.02	0.80+/−0.01	0.78+/−0.01	0.90+/−0.01
Stiffness (N/mm)	158+/−34	300+/−48	92+/−25	38+/−14	300+/−27

day, as opposed to group 1, which had treatment applied to alternating sides each day. In hindsight, photodensity measurements should have been taken sooner and more frequently after pulsed US treatment began, as already at 1 week the different groups are substantially differentiated.

Vibratory Coherence Assessment

The vibratory coherence measurement was performed on all rabbits before the osteotomy was made and after dissection of the rabbits' mandibles. It was noted that in five rabbits, the coherence averaged over the range 0–800 Hz for the dissected (*in vitro*) mandible was higher than that for the coherence taken before the osteotomy (*in vivo*). Therefore, seven other normal rabbits' heads obtained from another study (those used in the mechanical testing) underwent the same testing before the mandibles were dissected, and after dissection. There was a 2% increase in the coherence averaged over the range 0–800 Hz after dissection (*in vitro*) relative to before dissection (*in vivo*). Thus, in retrospect, the data from all the coherence measurements after dissection of the mandibles in all samples were reduced by 2%. Table 1 and Fig. 3(b) show the mean and standard error of the coherence averaged over 0–800 Hz after distraction relative to before distraction in each group and also of the normal bone group.

Mechanical Stiffness Test

Table 1 and Fig. 3(c) show the mean and standard error of the measured mandible stiffness in all groups. Again, differences between the groups are similar to those observed using photodensity and vibratory coherence. A one-way ANOVA calculation and a posthoc multiple comparison test (Scheffe) indicated that the statistical differences were significant when comparing group 3 (the control) with the left side of group 2 (0.003 significance) and with normal bone (0.002 significance).

Histological Examination

In all sections, bone formation was examined on the outer surface of the mandible since deeper layers contained the growing parts of the lower incisors. (Note, it was observed that the teeth grew at a faster rate when subjected to pulsed US treatment, with the group 2 left side teeth growing the fastest. However, these teeth contained an immature woven bone-like structure. This phenomenon warrants further study and discussion in a separate article; it is not the focus here.)

Histological examination of the distraction site in group 1 [Fig. 4(a)] shows that the distraction zone was composed mainly of immature bone with some bone trabeculae observed in the middle of the distraction zone. Osteoblastic activity was evident as indicated by numerous osteoblasts that are lining the newly formed bony trabeculae which is indicative of active bone formation. The newly formed bone matrix exhibited numerous osteocytes in their lacunae. Bone lamellae were present in many sections, but no Haversian system-like configuration was observed within the distraction zone.

In group 2 on the left side, the distraction zone was filled mainly with mature lamellar type bone [Fig. 4(b)] with some scattered islands of woven bone. It can be seen that the newly formed bony lamellae are arranged in circular pattern around blood vessels making Haversian system-like configurations, which is an indication of bone maturation. Qualitative observations seem to indicate the presence of more osteocytes within the newly formed bone matrix when compared to similar regions in group 1. The boundary between the newly formed bone and old bone was hard to distinguish due to similar arrangement of the bony lamellae in both original and newly formed bone at the distraction site. Also, in most sections osteoclastic activity was seen which is indicative of ongoing bone remodeling.

For the right side of group 2 [Fig. 4(c)] the distraction zone is filled mainly by immature woven bone that shows apposition as indicated by the presence of numerous osteoblasts lining the newly formed bony trabeculae. Also osteocytes were observed within the bone matrix.

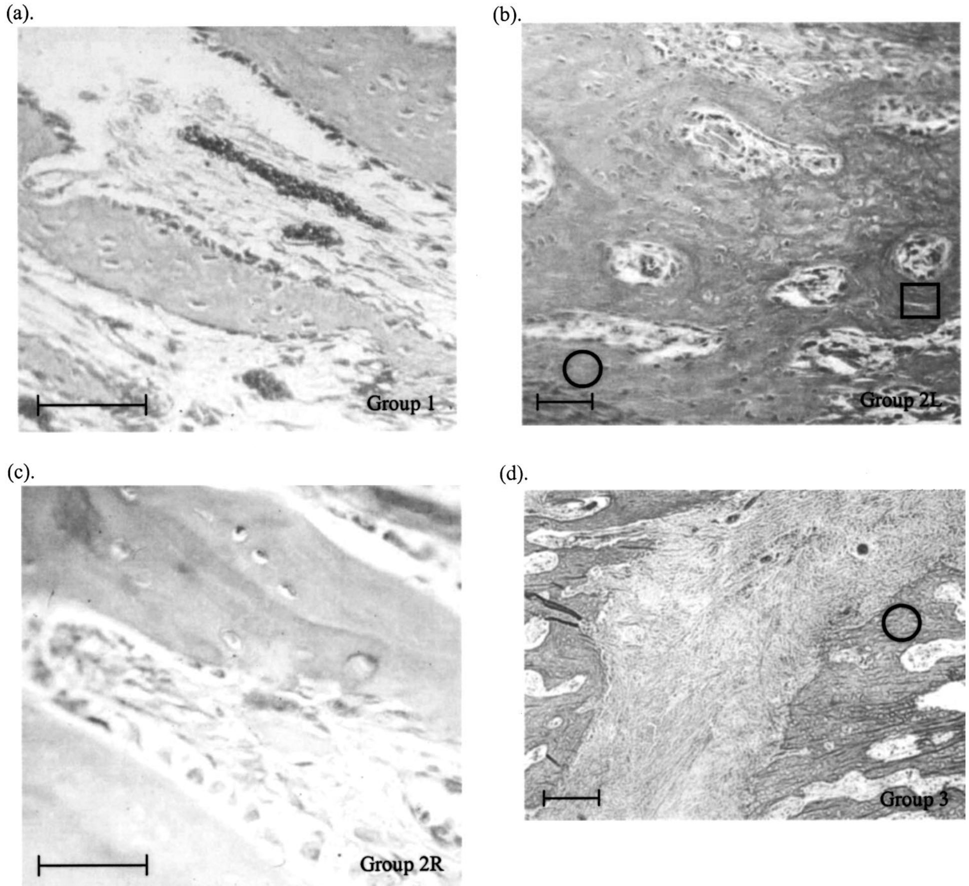

FIGURE 4. Histological section of the distraction site in: (a) group 1—pulsed US alternating sides every other day; (b) group 2 left side—direct pulsed US every day; (c) group 2 right side—indirect pulsed US every day; and (d) group 3—no pulsed US. Scale bars in all micrographs equal 100 μm. Circles indicate areas of woven bone. The square indicates an area of lamellar bone.

However, the osteoblasts and osteocytes observed in this group at the distraction site qualitatively appeared to be less prominent than those seen in comparable sections of group 1. Islands of chondrocytes were also seen around the middle of the distraction zone (not shown).

Group 3 histology: it can be seen that the middle of the distraction zone is filled with fibrous tissue with no evidence of bone [Fig. 4(d)]. Also, in group 3, qualitative observations indicated the presence of less osteoblastic activity than noticed in groups 1 or 2. Between the middle fibrous zone and the original bone at the boundary of the distraction site, there were scattered areas of fibrocartilage with numerous chondrocytes that may indicate endochodral bone formation/replacement. Next to the cartilaginous islands, woven bone was seen filling the remaining area of the distraction site next to the original bone of the mandible (not shown).

DISCUSSION

Pulsed Ultrasound Treatment Choices

Pulsed US was applied for 4 weeks after distraction since it was reported it has its greatest effect in stimulation of bone formation in the first 2–3 weeks of treatment.[28] Pulsed US application was different for groups 1, 2, and 3 to provide a more comprehensive evaluation of its effect after distraction of the mandible. The experimental design allowed such evaluation between group 1, which received treatment to both sides of the mandible every other day and the control, group 3, which received no treatment. Furthermore, in group 2, the response to direct treatment on one side of the mandible every day was compared to the contralateral side, which received only indirect treatment. Group 2 was also compared to group 1, which received direct treatment only every other day to a particular side. Previous bone healing studies after fracture have shown that the optimum benefits of using therapeutic pulsed US were gained after application of it for 15–20 min every day using a comparable power and frequency content to those used in this study for the direct treatment condition.[21,27–30]

There were only two previous research studies that the authors were aware of that reported on the use of pulsed US to enhance bone healing after osteodistraction.

In the first study,[24] two different groups of rabbits underwent osteodistraction of their right tibia at different rates, 0.5 mm per 12 h for 10 days and 1.5 mm per 12 h for 7 days. Half of each group received pulsed US treatment of 30 mW/cm^2 (with the same frequency content as the present study) for 20 min per day after ceasing distraction. For both groups, pulsed US treatment substantially accelerated bone maturation in the distracted region. In the second study,[18] callus distraction on the right metatarsus of 18 female mature merino sheep was performed at a rate of 0.5 mm/12 h for 16 days. After distraction, half the sheep received daily pulsed US treatment using the same power level, frequency content, and duration as used in this and the other cited study. Analysis of the interzone relation and callus relation in high-resolution radiographs and bone mineral content measurements based on computed tomography scans showed significantly accelerated bone maturation in the pulsed US stimulated group.

Radiographic, Vibratory, and Mechanical Stiffness Measurements

Referring to Fig. 3 and Table 1, in addition to the differences between the three groups, there are substantial differences between the two sides of group 2. This difference and the difference with respect to group 1 suggest a dose-dependent level of osteogenesis. Direct treatment every day (group 2 left side) stimulates more growth than direct treatment every other day alternating with indirect treatment days (group 1), which in turn has a greater effect than indirect treatment every day (group 2 right side). The pulsed US energy level will decrease exponentially with tissue penetration; it is suspected that the energy input under direct treatment is substantially greater than under indirect treatment. An order-of-magnitude estimate of the degree of attenuation and relative dose strengths between the direct and indirect sides can be made based on the following assumptions: (1) plane wave propagation through one side of the jaw and mandible, through the tongue and into the other side of the jaw and mandible (in reality, there will be additional loss due to scattering, especially with the presence of air regions); and (2) the pressure attenuation coefficient for teeth/mandible is $\alpha_{\text{bone}}=1.5$ Np/cm and for the tongue is $\alpha_{\text{tongue}}=0.15$ Np/cm at 1.5 MHz[9,10] assuming the bone thickness is 0.5 cm and the tongue thickness is 2 cm. With these assumptions the fraction of the intensity reaching the other side of the jaw would be

$$\begin{aligned} I/I_0 &= \exp[-2\alpha_{\text{bone}}d_{\text{bone}}]\exp[-2\alpha_{\text{tongue}}d_{\text{tongue}}] \\ &= \exp[-2(1.5)(0.5)]\exp[-2(0.15)(2)] \\ &= 0.122, \end{aligned} \tag{4}$$

TABLE 2. Comparison between groups of the photodensity of the newly formed bone to the normal bone next to distraction site by the ANOVA test at the end of each week after distraction. (*The mean difference is significant at the 0.05 level.)

Weeks after distraction	Comparison	Significance
1	1 vs 2	0.24
″	1 vs 3	0.91
″	2 vs 3	0.09
2	1 vs 2	0.52
″	1 vs 3	0.11
″	2 vs 3	0.01*
3	1 vs 2	0.23
″	1 vs 3	0.17
″	2 vs 3	0.006*
4	1 vs 2	0.69
″	1 vs 3	0.01*
″	2 vs 3	0.002*
4, Left side	1 vs 2	0.31
″	1 vs 3	0.02*
″	2 vs 3	0.001*
4, Right side	1 vs 2	0.116
″	1 vs 3	0.04*
″	2 vs 3	0.88
4	2-Lt vs 2-Rt	0.003*

where I is the intensity at the other side and I_0 is the intensity incident on the first side. This is a very coarse estimate that may be taken as an upper bound; but, it predicts that 12.2% of the energy gets to the far (indirect) side relative to the near (direct) side.

Table 2 shows the one-way ANOVA calculations with Scheffe analysis that give a measure of the significance of the statistical differences in photodensity percentages between the groups. There is a consistent significant difference between group 3, the control, and group 2, which received daily, pulsed US treatment to one side. Differences between groups 1 and 3 also eventually become significant after 4 weeks. These results suggest that there may be an upper bound on the potential effect of the pulsed US treatment that was reached sooner by the higher dosage group (group 2 left side) and later by the lower dosage group (group 1). However, further studies are needed to confirm this hypothesis.

With respect to vibratory coherence measurements, it can be seen that there is a pattern similar to that seen for the photodensity. ANOVA calculations indicated that the statistical differences were significant at the 0.05 level when comparing group 3 (the control) with the left side of group 2 (0.001 significance) and with normal bone (0.001 significance). When comparing group 3 with group 1 a value of $p=0.08$ was found, suggesting a trend but not establishing statistical significance.

With respect to the mechanical stiffness test, it appears that group 2, left side, which received the daily direct, pulsed US treatment, has, within statistical significance, attained a bending stiffness value that is comparable to that of normal bone.

Histological Observations

The configuration found in group 1 animals (alternating direct and indirect pulsed US treatment) is in agreement with findings of Komuro *et al.*[16] who reported a similar histological picture in the rabbit's mandible after 4 weeks of distraction at a rate that was slower than in the present study (0.18 mm each 12 h for 24 days). The presence of bone lamellae in the distraction area was not reported by these authors; but, it is in agreement with Hagiwara and Bell[12] who used a distraction rate of 0.7 mm per day for 10 days and found a similar appearance after 20 days of stabilization and electrical stimulation of the bone. This suggests that the pulsed US treatment had an effect similar to the electrical stimulation applied in that study.[12] The histological configuration found in group 2 animals on the left side (daily direct application of pulsed US) is in agreement with the findings of Komuro *et al.*[16] who reported a similar pattern of bone healing and formation at 6–8 weeks after distraction. This suggests that daily application of direct pulsed US could accelerate bone formation and maturation more than direct pulsed US treatment every other day. In group 3 animals (no pulsed US treatment) the presence of the middle fibrous zone and the scattered areas of fibrocartilage are in agreement with the findings of Stewart *et al.*[26] who reported a similar histological pattern in the rapidly distracted group (3 mm/day). This pattern has also been described in Komuro *et al.*,[16] which reported similar findings 2 weeks after distraction. This may be due to the difference in the latency period (2 weeks in the study of Komuro *et al.*)[16] as well as the distraction rate (0.36 mm/day).

Possible Explanations for Results

The mechanism or mechanisms for enhanced osteogenesis due to pulsed US stimulation at fracture sites, as well as distraction sites, is not well understood. The present study does not address the underlying cause, but rather provides further evidence of its effect in the case of mandibular distraction. It has been hypothesized that the stimulatory effect of pulsed US may be due to the increased formation of new blood vessels,[31] the increased secretion of prostaglandin E2,[27] the increased secretion of growth factors,[14] and/or may be related to the piezoelectric properties of biological tissue.[3] Electric potential produced by pulsed US via the piezoelectric effect may increase bone formation and accelerate bone healing.[12,32] Alternatively, electromagnetic stimulation may result in therapeutic mechanical stimulation via the piezoelectric effect; i.e., pulsed US (mechanical) stimulation may be a more ''direct'' therapy than electrical stimulation.

Another unanswered question may be whether the therapeutic effect is specifically dependent on the frequency content of the applied vibratory stimulation. The pulsed US treatment used in this and many studies consists of two frequencies, one at the fundamental thickness resonance (1.5 MHz) of the piezoelectric transduction probe, and one at the pulse repetition rate (1.1 kHz) applied to the transduction probe. Arguably, this choice of frequencies is more a matter of convenience than rigorous scientific investigation. Probes producing frequencies in this range are abundantly available due to their application in pulse-echo ultrasonic imaging. Perhaps the therapeutic effect of pulsed US was first fortuitously observed as a result of medical imaging protocols with such probes. The relative importance of the 1.1 kHz and 1.5 MHz components at certain amplitude levels, frequencies or power levels is all material for further research. Indeed, there have been a number of ''nonultrasonic'' vibratory studies that have yielded similar osteogenic results using cyclic loading frequencies as low as 0.2 Hz (e.g., Ref. 15). It has been hypothesized that the cause of the osteogenic effect under cyclic mechanical loading at very low frequencies is due to the enhanced interstitial fluid flow in the bone, which elicits a functional adaptation response in the bone at the cellular level. The biophysical mechanisms underlying this response may include direct or indirect mechanical effects, electromechanical effects, and enhancement of molecular transport. So then, it may be a question of what type of vibratory stimulation best initiates interstitial fluid flow, or more fundamentally the required mechanical and electromechanical effects or molecular transport. It is conceivable that a wide range of infrasonic, sonic, ultrasonic, or combination stimuli may be able to produce a useful combination of these mechanisms. Indeed, there may be more than one mechanism at work here, with different mechanisms being prevalent for different dynamic loading frequencies and modalities.

CONCLUSION

The following conclusions are based on the radiographic, vibration, mechanical, and histological results of the present study.

(1) Daily direct application of pulsed US during the 4 weeks after distraction accelerated bone formation and maturation when evaluated by photodensitometric, vibratory, elastic, and histological techniques.
(2) Direct pulsed US application every other day alternating with indirect application increased bone formation and growth, but not to the same extent as direct application of pulsed US every day, which may indicate that the stimulatory effect is dose dependent.

(3) Further studies are needed to confirm these results with a larger sample size, and to investigate the molecular or cellular basis of the role of pulsed US and other vibratory stimuli in enhancing bone formation at the distraction site.

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