Journal of Orthopaedic Trauma
Vol. 4, No. 3, pp. 246–253

Non-Invasive Low-Intensity Pulsed Ultrasound Accelerates Bone Healing in the Rabbit

A. A. Pilla, M. A. Mont, P. R. Nasser, S. A. Khan, M. Figueiredo, J. J. Kaufman, and R. S. Siffert

Bioelectrochemistry Laboratory and the Carroll and Milton Petrie Orthopaedic Research Laboratory, Department of Orthopaedics, Mount Sinai School of Medicine, New York, New York, U.S.A.

Summary: The effect of ultrasound (US) on the rate of fibula osteotomy healing in 139 mature New Zealand white rabbits was assessed in this study. Bilateral midshaft fibular osteotomies were made using a 1-mm Gigli saw. US was noninvasively applied to one limb for 20 minutes daily, while the contralateral limb served as a control. A 2.5-cm PZT transducer was applied to both limbs, with the treated limb receiving a 200-μs burst of 1.5-MHz sine waves repeated at 1.0 kHz. The incident intensity was approximately 30 mW/cm^2. Animals were killed at intervals between 14 and 28 days. Maximum strength increases (significant to $p \leq 0.01$) ranged from 40 to 85% from postoperative day 14 to 23. On day 28, no significant difference in ultimate strength was noted. From day 17 through day 28, all US-treated fractures were as strong as intact bones ($p \leq 0.005$). On the other hand, the ultimate strength of the control osteotomies attained intact values only by day 28. These results indicate that biomechanical healing is accelerated by a factor of nearly 1.7. This occurs with an overall acceleration of the healing curve in this fresh fracture model. If noninvasive low-intensity pulsed sine wave ultrasound can significantly accelerate bone repair in clinical application with an in-home treatment of 20 minutes daily, then US may be a useful adjunct for fracture care with a concomitant impact on patient morbidity. **Key Words:** Ultrasound—Fracture healing—Rabbit fibula—Biomechanical testing.

There are many applications of ultrasound (US) in surgery, therapeutics, and diagnostics (47). The frequency range employed is 0.8 to 15.0 MHz and is based on considerations of sound absorption, penetration, and resolution (for diagnostics). The intensities are high for surgical and therapeutic applications (1–50 W/cm^2), and are designed to cause significant tissue heating. Diagnostic US employs much lower intensities (1–50 mW/cm^2), which are specifically chosen to avoid tissue heating. Low-intensity ultrasound in the latter power range has recently been used in an attempt to assess the mechanical state of bone in a healing fracture (16) and various stages of osteoporosis (2,17). Weak US has been shown to influence the rate of fracture repair in animal models (11,13) and bone repair in humans (3,22,44,45). Several types of experimental animal fractures have been studied, using widely differing doses and signals of ultrasound (1,14,20–22,26). Assessment of bone repair in these studies has been carried out primarily by radiographic analyses. To the authors' knowledge there has been no well-controlled study of the influence of US on the rate of return of the mechanical strength of bone in a healing fracture. Preliminary studies in this labora-

Address correspondence and reprint requests to Arthur A. Pilla, Ph.D., Department of Orthopaedics MS 1188, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, U.S.A.

tory have shown that a non-thermal US signal can accelerate fracture healing in rabbit fibula osteotomy (28,30,32,33). A similar pulsed US signal has been used in double-blind placebo-controlled clinical studies of Colles' and tibia diaphyseal fractures and resulted in an acceleration of healing (as demonstrated by radiography) at all time points by an average factor of 1.5 (24). This work reports on a quantitative time study of the effect on bone repair of low-intensity US (30.0 mW/cm^2, non-thermal) at 1.5 MHz in a model of bilateral rabbit fibula osteotomy, using biomechanical assessment of bone strength.

MATERIALS AND METHODS

Operative Technique

The experimental model consisted of bilateral midshaft fibular osteotomies performed on 139 mature (3.5–5 kg) female New Zealand white rabbits (6). Under aseptic conditions using xylazine (20 mg/ml) and ketamine (50 mg/ml) as anesthesia, the hind limbs were shaved from mid-thigh to ankle, and a depilatory agent was employed to maintain the skin fur-free for efficient ultrasound coupling during treatment. A lateral approach was used to reach the fibula. With the distal tibial tuberosity as a central landmark, a 4-cm incision was made through the skin and fascial layers. The plane between the gastrocnemius/flexor hallucis longus and the tibialis cranialis was separated using blunt dissection until the tendons of the extensor digitorum longi were exposed. Curved elevators were then passed through the interosseous membrane, separating the tibia and fibula at 2 cm from the tibial tuberosity (midfibular shaft). A 1-mm Gigli saw was aligned perpendicularly to the fibular longitudinal axis, and with gentle, short strokes, the fibula was osteotomized under constant saline irrigation. The osteotomy was complete when the Gigli saw had passed two-thirds through the fibular diameter and the distal and proximal ends were separated along a scalpel blade score on the lateral aspect. Fractures were accepted only if they were in the middle third, were not comminuted, had bone contact in the lateral gap portion, and were not displaced greater than 25% of the bone diameter. The skin and fascia were closed with 4.0 subcuticular coated vicryl sutures. An identical procedure was performed on the contralateral side. The fibula is synostosed to the tibia proximally and distally, and thus required no fixation. To avoid surgical bias, operative procedures on consecutive animals were always done alternately from left to right limbs. The rabbits were allowed to resume unrestricted activity in their cages upon recovery from anesthesia. A schematic diagram of the lateral view of the tibia and osteotomized fibula is illustrated in Fig. 1.

Treatment Groups

All animals in this study were placed in standard sling immobilization (25) for a 20-minute treatment period starting on postoperative day (POD) 1. For the remainder of each 24 hours, the rabbits were allowed unrestricted activity in Department of Agriculture–approved cages.

The experimental animal group received 20-minute US treatments daily on one limb, between 5 and 10 p.m., while the opposite limb served as the contralateral control. US treatment started on POD 1 and was applied with a lead zirconate titanate (PZT-4) transducer (Interpore Orthopaedics Inc., Spring Valley, NY), 2.5 cm diameter, placed on the limb laterally over the osteotomy site (Fig. 1). Basic

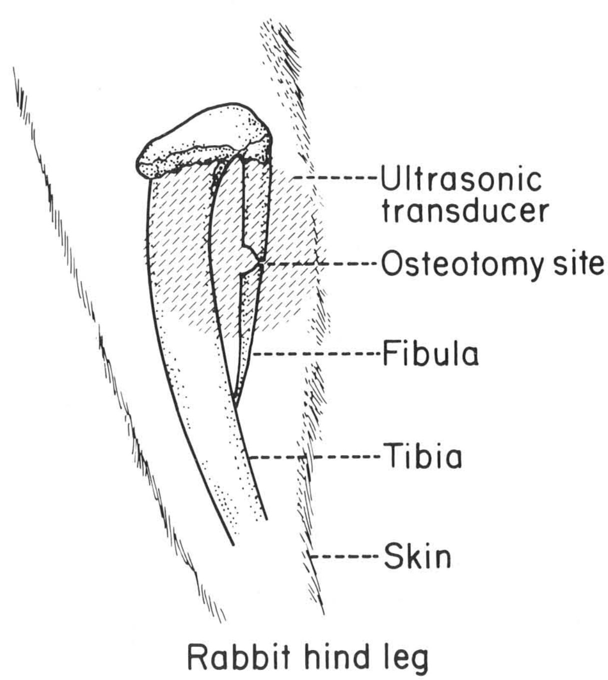

FIG. 1. Schematic diagram of the lateral view of the tibia and osteotomized fibula. The osteotomy is made in a "C" shape to provide bone contact and maintain a relatively stable healing curve. Note that the ultrasound transducer completely encompasses the osteotomy site.

ultrasound gel served as the coupling medium. A dummy transducer was applied to the contralateral limb, also using gel. US-treated animals were killed at PODs 14, 16, 17, 18, 21, 23, and 28, which encompasses all the gross structural stages of fracture healing (43) in this fibula model (5,6). Destructive torsional testing could not reliably be performed prior to POD 14 since most of the specimens exhibited only early soft tissue stage I healing (43).

There were 20 animals in the bilateral control groups that received complete osteotomies. They were handled on a daily basis in exactly the same manner as the US-treated animals. Dummy transducers were applied to both limbs for 20 minutes daily, using ultrasound gel. These animals were killed at postoperative day 17 and 18. To obtain intact specimens, 13 animals were selected at random times throughout the study.

The Ultrasound Signal

The low-intensity US signal used in this study was first described by Duarte, who reported successful results in animal models (1) and human clinical trials (44,45). The signal consisted of a 200 μs burst of 1.5 MHz sine waves repeating at 1.0 kHz and delivering 30.0 ± 5.0 mW/cm^2 spatial average and temporal average incident intensity (SATA) (12,23). The effective radiating area of the PZT-4 transducer was 3.88 cm^2. The US signal was transmitted through the skin and intervening soft tissue by applying the transducer to the external skin surface via coupling gel. The relatively low soft-tissue attenuation of the US signal having a carrier frequency of 1.5 MHz allowed a significant portion of the signal to reach the fracture site (42). The signal was applied in a burst rather than continuously to obtain a ratio between peak amplitude and average power such that cavitation (41) and tissue heating were avoided (40). To check for the latter, in vivo temperature was monitored during US application by implanting a thermistor (YSI model 401) adjacent to the osteotomy site. The US signal was applied for 20 minutes in each 24-hour period, which is consistent with reported exposure times for mechanical effects on bone tissue in vitro (7,15) and in vivo (35).

Radiographic Evaluation

All specimens were examined radiographically after death and dissection using a Faxitron system (Hewlett Packard model 43805N), with an extremity cassette for high-resolution films. The radiographs were digitized (9,38) and evaluated to assure that each fibula pair had similar osteotomies. This was determined by accepting only "C" shaped osteotomies, i.e., those in which the Gigli saw had passed two-thirds through the thickness of the fibula (Fig. 2). These radiographs were also employed to make certain that the osteotomy was in the middle third and that left and right were not unequally displaced. A sample radiograph for POD 17 is shown in Fig. 2.

Destructive Torsional Testing

All fibulae were subjected to destructive torsional testing (courtesy A. H. Burstein, Hospital for Special Surgery, New York, NY). Specimens were prepared for mechanical testing by cutting the tibia on

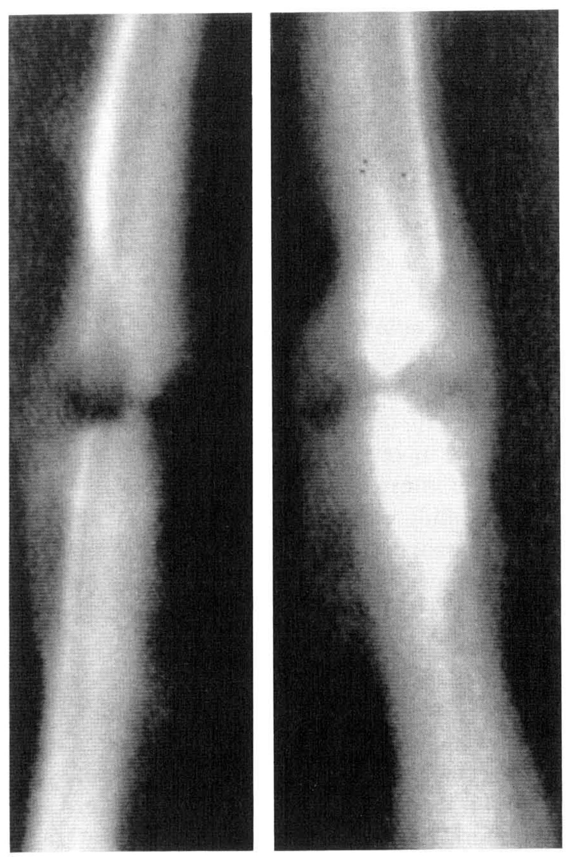

FIG. 2. Radiograph for POD 17. US-treated side (right) exhibits more mature callus than its contralateral control. The US-exposed bone is as strong in torsion as intact fibulae, while the contralateral control is still only about 50% of intact strength (Table 1). Note the more extensive periosteal reaction on the right.

a diagonal, beginning at the ridge separating the medial and lateral condyles and proceeding along the lateral side of the tuberosity toward the medial surface. This method removes a wedge approximately 3 cm long and enables easy alignment of the fibula with the axis of applied torque. The exposed ends of the tibia were then embedded in Woods metal (Cerrobend alloy, melting temperature 70°C). The potted specimen was placed in the dead-weight testing apparatus (8), and the tibia was isolated proximal to the osteotomy at the region of maximal separation of the tibia and fibula using a small power saw (Dremel Moto-Tool with 1.0 × .005 in blade). With the specimen in place, the torque versus angle curve was recorded. A typical torque versus angle curve is shown in Fig. 3. Maximum torque is the peak of the curve immediately prior to specimen failure, expressed in newton meters (Nm). Stiffness was calculated for each sample by taking the slope of the longest straight line, starting at the origin, such that the standard error of regression was within the experimental error of $\pm 5.0 \times 10^{-4}$ Nm (Fig. 3). The biomechanical results are reported as maximum torque and initial torsional stiffness.

Statistical Analysis

The paired percentage difference of each treated fibula relative to its contralateral control was calculated as 100 times (US treated value minus control value) divided by the control value. The mean and standard error of the mean (SEM) was computed for each POD. All paired percentage differences in maximum torque and derived initial torsional stiffness of the rabbit fibulae were tested for normality (Gaussian distribution) using a Kolmogorov-Smirnov goodness of fit test. A one-tailed Student's *t* test was employed to test the paired percentage differences for statistical significance. A two-tailed Student's *t* test was used to test the statistical significance of differences from intact strength for both treated and contralateral control osteotomies for each postoperative day. For both of the above cases significance was accepted at $p \leq 0.05$ (46).

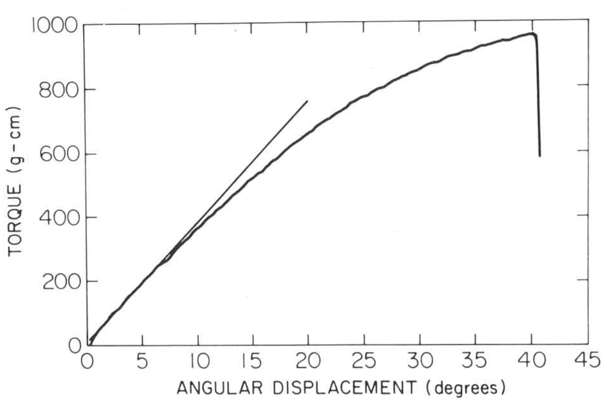

FIG. 3. A typical torque versus angular displacement curve for a rabbit fibula osteotomy exhibiting a relatively mature [stage III (43)] callus (typical of US-treated fibulae from POD 16 onward). The straight line tangent to the experimental curve starting at the origin is a measure of the torsional stiffness.

RESULTS

Of 139 starting animals, 106 were accepted for inclusion in the study. Of the original group, 12 were infected, and 21 had unequal gap osteotomies, were displaced, or were ruined during mechanical testing. The maximum temperature rise at the osteotomy site at the end of 20 minutes of US exposure was 0.1 ± 0.02°C, indicating that gross thermal effects were not a factor for this signal.

The healing curve, using maximum mechanical strength for the osteotomy model in the rabbit fibula, exhibits rapid transition of the fracture from soft callus to hard callus (stage I to stage II, [43]) within POD 14–23 (5). During this interval, failure in approximately 90% of all control osteotomies occurred in the callus. The mechanical test results exhibit the greatest difference for treated limbs versus contralateral controls in this time period. A summary of the mechanical strength data is presented for each POD in Table 1. Maximum strength differences compared with controls were most notable on day 14, 16, 17, 18, 21, and 23, when treated limbs were 40–85% stronger than their contralateral controls. On day 17 the treated fractures exhibited an average maximum torque of 9.88×10^{-2} Nm, which is not significantly different from 1.02×10^{-1} Nm, the average for intact fibulae ($p < 0.005$). On the other hand, it was only at POD 28, when they exhibited a mean torque of 1.22×10^{-1} Nm, that the mean strength of control limbs was not significantly different ($p < 0.005$) from intact values. Bilateral control results for POD 17 and 18 (Table 1) showed no significant paired percentage effect. The maximum torque values for these control fibulae are identical to those obtained for the contralateral control osteotomies of US-treated animals on the same POD. This suggests that surgical bias and/or systemic effects do not play a significant role in the observed US effect.

TABLE 1. *Ultrasound effect on maximum torque (Nm ± SEM) (×10⁴) in rabbit fibula osteotomy*

POD	Contralateral control	Treated	Average paired percent effect	n	p
14	415 ± 70	488 ± 70	67	13	<.05
16	437 ± 58	597 ± 80	45	8	<.025
17	593 ± 82	988 ± 136	85	12	<.0005
18	558 ± 81	839 ± 88	86	12	<.01
21	655 ± 90	924 ± 122	59	11	<.01
23	660 ± 75	908 ± 154	38	8	<.03
28	1223 ± 130	1136 ± 111	−4	9	NS
	Bilateral controls				
	left	right			
17	620 ± 55	607 ± 69	−3	12	NS
18	567 ± 106	550 ± 85	4	8	NS
	Intact controls				
	left	right			
	957 ± 72	1088 ± 102	14	13	NS

POD, postoperative day; n, number of animals.

The influence of US on the healing curve is most clearly seen by comparing the strength of both treated and contralateral controls with that of the mean for intact fibulae at each POD. This is shown in Fig. 4, wherein the asterisked bars represent those groups that have maximum strengths that are significantly different from intact fibulae. As can be seen, the US-treated osteotomies achieve intact strength at POD 17 and remain at intact values for the remainder of the healing time tested (to POD 28). In contrast, the contralateral controls are significantly weaker than intact fibulae until POD 28. These results indicate that US increases the overall healing rate in this model by a factor of nearly 1.7.

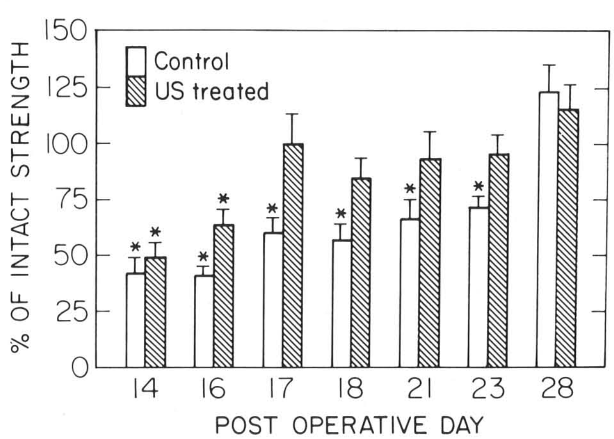

FIG. 4. Comparison of maximum strength of both US-treated and contralateral control limbs versus intact fibulae for each POD. The US-exposed limbs reach intact strength at POD 17 and maintain this strength for the remainder of the test period. The contralateral control fibulae reach intact strength only by POD 28. Significantly different than intact fibulae *p < 0.005.

The average torsional stiffness was calculated using each torque versus angle curve by fitting the longest straight line from the origin consistent with an experimental error of $\pm 5.0 \times 10^{-4}$ Nm in torque (Fig. 3) and is shown in Table 2 for each POD.

DISCUSSION

It has been shown in this study that low-intensity pulsed sine wave US noninvasively applied for 20 minutes daily increased the rate of return of mechanical strength of an osteotomized rabbit fibula, as assessed by destructive torsional testing. Both initial stiffness and maximum torque exhibited significant differences for the US-exposed fibula versus its contralateral control during the period of rapid callus maturation. The US effect was intense enough during the mineralization phase to affect the entire healing curve. Thus the treated osteotomy achieved intact strength approximately 1.7 times as fast as its contralateral control. For the US-treated fibulae, stiffness and maximum strength returned to normal (intact values) at the same rate (see Tables 1 and 2). On the other hand, for the contralateral controls, stiffness returned to intact values at a somewhat faster rate than maximum strength (POD 23 versus POD 28), confirming the findings of previous studies of healing fractures (5). The stiffness at POD 28 is considerably higher (for both treated and control sides) than that for intact values. This finding may be due to the fact that the average callus size in the area of the osteotomy site nearly doubles the effective diameter of the bone and therefore multi-

TABLE 2. *Ultrasound effect on torsional stiffness (Nm/deg ± SEM) (×10^4) in rabbit fibula osteotomy*

POD	Control	Treated	Average paired percent effect	n	p
14	8 ± 2	10 ± 2	91	13	<.05
16	9 ± 2	14 ± 2	70	8	<.025
17	13 ± 2	20 ± 2	82	12	<.005
18	12 ± 2	19 ± 2	86	12	<.002
21	15 ± 2	20 ± 3	45	11	<.02
23	21 ± 4	23 ± 5	27	8	NS
28	42 ± 7	37 ± 5	17	9	NS
	Bilateral controls				
	left	right			
17	12 ± 2	12 ± 2	10	12	NS
18	14 ± 4	12 ± 3	2	8	NS
	Intact controls				
	left	right			
	23 ± 2	24 ± 2	3	13	NS

POD, postoperative day; n, number of animals.

plies its moment of inertia by a factor of 16. Since stiffness in this torque test may be considered to be a summation of its values for each cross section of the bone along its entire length and since mature callus occupies the middle third of the fibula, the resulting structure must have greater overall stiffness than the intact bone. Maximum strength of treated and control bones is not significantly different from that of the mean of intact values, despite the high stiffness value at POD 28. This is most probably due to the fact that the fibula fails outside the callus in the shaft with mechanical strength similar to that of the intact shaft. This corresponds to late stage III and stage IV fracture healing (43). Support for this theory may be seen in the data of Table 2, which shows that treated fibulae do not exhibit a significant increase in maximum strength versus intact bones beyond POD 17, whereas, radiographically, the US-treated osteotomy generally shows a more mature and larger callus than its contralateral control (Fig. 2).

The results presented in this study show that low-intensity pulsed US is capable of accelerating the overall biomechanical healing rate in a controlled fracture model. It is important to correlate this finding with the actual biomechanical healing stage (43) of each fibula. The healing stage was determined by examination of each torque versus angle curve and by observation of the point of failure (callus, shaft, or both). This analysis showed that *none* of the US-treated fibulae exhibited soft-tissue behavior (stage I, [43]) from POD 17 through POD 28. In particular, from POD 17 onward, the majority of US-treated fibulae were in the hard tissue stages III and IV (43). For example, 58% were in stage III at POD 17, wherein the maximum torque strength was largely determined by that of the shaft. The mean strength of these fibulae was therefore not expected to be significantly different from intact (shaft) values, as is shown in Fig. 4. In contrast, the majority of contralateral control fibulae were in soft tissue stage I (53% at POD 17) or early hard tissue stage II from POD 17 through POD 23. The mean strength of these controls was expected to be determined by that of the callus as it progresses from stage I through stage II. The maximum strength should therefore be slowly rising and not yet equal to intact values, as seen in Fig. 4. The correlation presented above is a strong indication that the strength measurements for this bone correspond to a healing stage that is meaningful in terms of return of function.

Bone tissue is able to respond functionally to mechanical input, but the exact mechanism of this effect is not yet known. Proposed mechanisms center on direct and indirect mechanical effects. For example, it has been reported (4) that bone cells respond to static mechanical forces by an increase in prostaglandin (PGE_2) synthesis and that this is a direct result of the deformation of membrane phospholipid (via phospholipase A_2). Using US creates a pressure wave that may deform the cell membrane and alter ionic permeability (10) and therefore second messenger activity (29) via a direct mechanical effect. It is also possible that an indirect electrical effect, such as modulation of electrical charge and/

or ion binding at the membrane surface, could occur (18,19,27,29,34,39). This electrochemical perturbation may trigger a follow-up biological mechanism involving second messenger activity. Exposure of MC3T3 osteoblasts to the US signal used in this study showed an early (3, 5, and 15 min) effect on adenylate cyclase activity (31,36,37), suggesting that a biochemical cascade is triggered by low-intensity US.

The results of this in vivo animal study suggest that low-intensity pulsed ultrasound may be useful for bone repair and new bone formation as might occur in the biologic fixation of prosthetic implants, bone graft incorporation, allographic transplantation, and for osteonecrotic conditions. It is our belief that the physical modulation of biological systems using non-thermal pulsed ultrasound may bring a new and powerful approach to many clinical problems.

Acknowledgment: The authors wish to thank Kingsley Gyebi sincerely for his untiring dedication to this work, and Sebastian Lattuga, M.D., Mark Creighton, M.D., Mark Klion, M.D., Kenneth Hess, and James Ryaby for their significant contributions. Partial support from NIH Grant 5T35DK07420 and the PRIME foundation is gratefully acknowledged.

REFERENCES

1. Amelin AZ, Lotsova EI, Petukhova LI: Morphological characteristics of bones regenerated under the effect of ultrasound. *Mekhanika Polimerov* 6:1121–1122, 1978.
2. Baran DT, Kelly AM, Karellas A, et al.: Ultrasound attenuation of the os calcis in women with osteoporosis and hip fractures. *Calcif Tissue Int* 43:138–142, 1988.
3. Bernau A, Kruppa G: Niederfrequency- und Ultraschallbehandlung: Prospektive Studie uber 1200 Behandlungssequenzen. *Z Orthop* 119:126–137, 1981.
4. Binderman I, Zor U, Kaye AM, Shimshoni Z, Harell A, Somjen D: The transduction of mechanical force into biomechanical events in bone cells may involve activation of phospholipase A_2. *Calcif Tissue Int* 42:261–266, 1988.
5. Black J, Perdigon P, Brown N, Pollack SR: Stiffness and strength of fracture callus: Relative rates of mechanical maturation as evaluated by a uniaxial tensile test. *Clin Orthop Rel Res* 182:278–288, 1988.
6. Brighton CT, Hozack WJ, Brager MD, Windsor RE, Pollack SR, Vreslovic EJ, Kotwick JE: Fracture healing in rabbit fibula when subjected to various capacitively coupled electrical fields. *J Orthop Res* 3:331–340, 1985.
7. Buckley MJ, Banes AJ, Levin LG, et al.: Osteoblasts increase their rate of division and align in response to cyclic, mechanical tension in vitro. *Bone and Mineral* 4:225–236, 1988.
8. Burstein A, Frankel V: A standard test for laboratory animal bones. *J Biomech* 4:155–158, 1971.
9. Castleman KR: *Digital Image Processing*. Englewood Cliffs, New Jersey, Prentice Hall, 1979, pp 90–92.
10. Chapman IV, MacNally NA, Tucker S: Ultrasound induced changes in rates of influx and effux of potassium ions in rat thymocytes in vitro. *Ultrasound Med Biol* 6:47–58, 1979.
11. Duarte LR: The stimulation of bone growth by ultrasound. *Arch Orthop Trauma Surg* 101:153–159, 1983.
12. Duck FA, Starritt HC, Aindow JD, Perkins MA, Hawkins AJ: The output of pulse-echo ultrasound equipment: A survey of powers, pressures and intensities. *Brit J Radiol* 58:989–1001, 1985.
13. Dyson M, Brookes M: Stimulation of bone repair by ultrasound. *Ultrasound Med Biol* 8:61–66, 1982.
14. Goldblat VI: Processes of bone tissue regeneration under the effect of ultrasound: Experimental study. *Orthopediia Traumatologiia I Protezirovanie* 30:57–61, 1969.
15. Hasegawa S, Sato S, Saito S, Suzuki Y, Brunette D: Mechanical stretching increases the number of cultured bone cells synthesizing DNA and alters their pattern of protein synthesis. *Calcif Tissue Int* 37:431–436, 1985.
16. Horn CA, Robinson D: Assessment of fracture healing by ultrasonics. *J Coll Radiol Aust* 9:165–167, 1965.
17. Heaney RP, Avioli LV, Chestnut III CH, Lappe J, Rocker RR, Brandenburger GH: Osteoporotic bone fragility: Detection by ultrasound transmission velocity. *JAMA* 261:2986–2990, 1989.
18. Hrazdira I, Adler J: Electrokinetic properties of isolated cells exposed to low levels of ultrasound. In: *Ultrasound Interactions in Biology and Medicine,* ed by R Millner, E Rosenfeld, and U Cobet. New York, Plenum, 1983, pp 167–169.
19. Kaufman JJ, Popp H, Chiabrera A, Pilla AA: Effect of ultrasound on the impedance of biological cells. *Trans BRAGS* 8:8, 1988.
20. Klug W, Knoch HG: Durch biophysikalische Untersuchungen Quantifizierung der Knochenbruchheilung Nacl. Ultraschallstimulation von distalen Radiusfrakturen. *Beitr Orthop Traumatol* 33:384–391, 1986.
21. Klug W, Franke W-G, Knock H-G: Scintigraphic control of bone fracture healing under ultrasonic stimulation: An animal experimental study. *Eur J Nucl Med* 11:494–497, 1986.
22. Klug W: Stimulierung de Knochenbruchheilung durch Ultraschall: Tierexperimentelle Studie. *Beitr Orthop Traumatol* 30:670–680, 1983.
23. Krembau FW: Physical considerations. In: *Biological Effects of Ultrasound,* ed by WL Nyborg and MC Ziskin, New York, Churchill Livingston, 1985, pp 14–16.
24. Kristiansen T, Pilla AA, Siffert RS, et al.: A multicenter study of the acceleration of Colles' fracture healing by noninvasive low intensity ultrasound. AAOS 57th Meeting, New Orleans, Feb. 1990.
25. Kumar A, Wong DA, Johnson RG, Herbert MA, Salter RB: The restraint of rabbits in a special sling. *Lab Anim Sci* 29:512–514, 1978.
26. Lotsova EI, Amelia AZ: An experimental study of ultrasonic influence upon the healing of bone lesions. *Kuroortologiia I Fizioterapiia* 14:20–22, 1977.
27. Miller DL, Nyborg WL, Whitcomb CC: Platelet aggregation induced by ultrasound under specialized conditions in vitro. *Science* 205:505–507, 1979.
28. Mont MA, Pilla AA, Tenreiro RA, et al.: The effects of ultrasonic stimulation on fresh fracture repair in rabbits. *Trans Orthop Res Soc* 12:97, 1987. (Note: The values of maximum torque reported herein are erroneous by a factor of two because of an undetected calibration error.)
29. Pilla AA, Kaufman JJ, Ryaby JT: Electrochemical kinetics at the cell membrane: A physicochemical link for electromagnetic bioeffects. In: *Mechanistic Approaches to Interactions of Electric and Electromagnetic Fields with Living Systems,* ed by M Blank and E Findl, New York, Plenum, 1987, pp 39–62.
30. Pilla AA, Khan SA, Nasser PR, Kaufman JJ, Siffert RS:

Low power ultrasound accelerates fracture healing in the rabbit fibula. *Trans BRAGS* 8:52, 1988.
31. Pilla AA, Ryaby JT, Figueiredo M, Nasser PR, Kaufman JJ, Siffert RS: Low intensity pulsed ultrasound modulates osteoblast activity and accelerates fracture repair in a rabbit model. *J Bone Min Res* 4:S255, 1989.
32. Pilla AA, Khan SA, Nasser PR, Kaufman JJ, Siffert RS: Low intensity pulsed ultrasound accelerates fracture repair in a rabbit model. *Trans Orthop Res Soc* 14:591, 1989.
33. Pilla AA, Figueiredo M, Nasser PR, et al.: Pulsed sine wave ultrasound: A potent accelerator of fresh fracture repair. *Trans BRAGS* 9:18, 1989.
34. Pilla AA, Figueiredo M, Lattuga S, Nasser PR, Alves JM, Kaufman JJ, Siffert RS: Pulsed sine wave ultrasound accelerates bone fracture repair. Proc IEEE 1989 Ultrasonics Symposium, 1989.
35. Rubin CT, Lanyon LE: Osteoregulatory nature of mechanical stimuli: Function as a determinant for adaptive remodeling in bone. *J Orthop Res* 5:300–310, 1987.
36. Ryaby JT, Bachner EJ, Bendo JA, Dalton PF, Tannenbaum S, Pilla AA: Low intensity pulsed ultrasound increases calcium incorporation in both differentiating cartilage and bone cell cultures. *Trans Orthop Res Soc* 14:15, 1989.
37. Ryaby JT, Duarte-Alves P, Pilla AA: Clinically effective ultrasound affects second messenger activity in bone cell cultures. *Trans BRAGS* 9:42, 1989.
38. Sartoris DJ, Sommer FG: Digital film processing: Applications to the musculoskeletal system. *Skeletal Radiol* 11:274–281, 1984.
39. Taylor KJW, Newman DL: Electrophoretic mobility of Ehrlich suspensions exposed to ultrasound of varying parameters. *Phys Med Biol* 17:270–276, 1972.
40. ter Haar GR, Hopewell JW: Ultrasonic heating of mammalian tissues in vivo. *Br J Cancer* 45:65–69, 1982.
41. ter Haar GR: Ultrasonic biophysics. In: *Physical Principles of Medical Ultrasonics,* ed by CR Hill, New York, Wiley & Sons, 1986, pp 379–427.
42. Wells PNT: *Biomedical Ultrasonics,* London, Academic Press, 1977, pp 120–123.
43. White AA, Panjabi MM, Southwick WO: The four biomechanical stages of bone repair. *J Bone Joint Surg* 59A:188–192, 1977.
44. Xavier CAM, Duarte LR: Estimulacão ultra-sonica de calo osseo: Aplicacão clinica. *Revista Brasileira de Ortopedia* 18:73–80, 1983.
45. Xavier CAM, Duarte LR: Treatment of non-unions by ultrasonic stimulation. AAOS 54th Meeting; Latin-American Orthopedic Association; San Francisco, Jan. 1987.
46. Zar JH: *Biostatistical Analysis.* Englewood Cliffs, New Jersey, Prentice Hall, 1984, pp 122–124.
47. Ziskin MC: Applications of ultrasound in medicine—comparison with other modalities. In: *Ultrasound: Medical Applications, Biological Effects and Hazard Potential,* ed by MH Repacholi, M Grandolfo and A Rindi, New York, Plenum, 1987, pp 49–61.