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D Pienkowski, SR Pollack, CT Brighton and NJ Griffith
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Low-Power Electromagnetic Stimulation of Osteotomized Rabbit Fibulae

A Randomized, Blinded Study*

BY D. PIENKOWSKI, PH.D.†, S. R. POLLACK, PH.D.‡, C. T. BRIGHTON, M.D., PH.D.‡, AND N. J. GRIFFITH, M.S.§, PHILADELPHIA, PENNSYLVANIA

Investigation performed at the University of Pennsylvania, Philadelphia

ABSTRACT: The purpose of this study was to determine whether low-power-consuming symmetrical-waveform electromagnetic stimuli could increase the stiffness of fracture sites in a rabbit fibular-osteotomy model. Both active and placebo devices were used in a blinded study protocol. Dose-response studies of pulse amplitude and pulse width were performed by continuous application (twenty-four hours a day) of repetitive (fifteen-hertz), bursted (five-millisecond-long) symmetrical, rectangular electromagnetic stimulus waveforms. The power consumed by these stimuli is approximately one-fifth that consumed by the pulsing electromagnetic field devices that are in current clinical use. Significant increase of callus bending stiffness was produced by pulse widths of five to seven microseconds and pulse amplitudes of fifty to 100 millivolts.

CLINICAL RELEVANCE: The large consumption of electrical power that is demanded by the specific waveform parameters of the pulsing electromagnetic fields used clinically necessitates an increase in the size, weight, and complexity of the devices, which in turn requires increased patient compliance and imposes an added management problem for the physician. Noncompliance on the part of the patient often causes loss of prescribed treatment time, and the possibility of therapeutic success is thereby diminished. The ideal stimulation device would be totally cast-incorporated; it would require no intervention on the part of the patient or physician, thereby ensuring delivery of the intended therapeutic dosage of electromagnetic stimulation; and its simplicity would facilitate the conduct of double-blind clinical-efficacy studies. The development of such a device requires the development of new specific parameters for electromagnetic stimuli that consume significantly less electrical power than do the current pulsing electromagnetic fields. Because the rabbit model used in the present study has previously been predictive of clinical success in the application of capacitively coupled stimulation for the treatment of fracture non-unions, it is expected that these specific signals have the potential to be clinically effective in the treatment of non-unions.

The use of specific pulsing electromagnetic fields for the treatment of non-unions is well known[4,15,36]. The advantages of pulsing electromagnetic fields, which include non-invasiveness and non-contact, are unmatched by other electrical-stimulation modalities, but substantial electrical power is needed to generate the signal used in clinical therapeutic devices. Although advances in battery technology and circuit design have eliminated the need for a 110-volt house current to power clinical delivery systems, the present devices are larger, heavier, and more complex than is ideal.

Because the stimulus signal waveforms that are used currently consume large amounts of electrical power, the patient must comply strictly with the instructions for recharging or replacement of the battery. This means that the administration of the therapeutic electromagnetic dosage is largely reliant on patient compliance, and inadequate stimulation time is possible due to noncompliance[3]. Two factors that have been associated with the failure of a stimulated fracture to heal are a patient's lack of understanding about the proper usage of the device and daily stimulation dosages that are less than the prescribed limits[9,35]; however, the percentage of patients in whom stimulation therapy fails for these reasons is unknown.

The ideal stimulation device would be small, lightweight, totally cast-incorporated, relatively inexpensive, and disposable. It would provide stimulation for as long

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†Division of Orthopaedic Surgery, K404 Kentucky Clinic, University of Kentucky, Lexington, Kentucky 40536-0284.

‡University of Pennsylvania, Philadelphia, Pennsylvania 19104. Please address requests for reprints to Dr. Brighton, Department of Orthopaedic Surgery Research, 424 Medical Education Building, Room 6081.

§LTI-IMD USA, Incorporated, 1637 Grant Road, Mountain View, California 94040.

as the cast remained intact and it would require no intervention by the patient or the physician. The simplicity of such a device would facilitate randomized double-blind clinical-efficacy studies, which are considered necessary for definitive proof of the effect of a mode of treatment[36]. Currently foreseen technological advances in battery capacity or in circuit or transducer refinement will not be able to achieve this ideal unless there is a substantial reduction in the electrical power consumed by the currently used stimulus signal.

A wide variety of specific electromagnetic-signal waveforms, some of which consume less power than others, have been demonstrated to produce biological effects[1,6,16,21,34]. We believed that there were other, as yet undiscovered, specific signal waveforms that can stimulate fracture-healing but that consume substantially less electrical power. Tests of this hypothesis began with our study of the pulse-amplitude and pulse-width components of the clinically used pulsing electromagnetic field[32], since these components offer the greatest opportunity for power savings.

The specific objective of this study was to determine the minimum pulse amplitude and pulse width of a symmetrical rectangular pulse burst that would significantly increase the stiffness of osteotomized rabbit fibulae.

Materials and Methods

Rationale for Selection of the Model

Healing in the New Zealand White rabbit fibular-osteotomy model has been characterized histologically, mechanically, and radiographically[5,18]. Although this model is a fresh fracture, it has been shown to be useful in the prediction of biological activity in the healing of non-unions in humans. Specifically, when the capacitively coupled sinusoidal electrical signal that was shown to be most effective in this model[10] was applied to the treatment of recalcitrant non-unions in humans in a multicenter clinical study, a beneficial therapeutic effect was observed[7]. Furthermore, this model allows comparison with previous studies that used direct electrical stimulation[18], electromagnetic stimulation[24,32,39], and ultrasound[33].

The stiffness of a healing osteotomized rabbit fibula increases sigmoidally with time[18]. The rate of change of fibular stiffness is greatest at approximately sixteen days after the osteotomy, the mid-point of healing. At this time, the fracture is the most responsive to the effects of the stimulation. Within the first eight days after the operation, fibular healing is not sufficient to allow for reliable testing, and after the third week of healing, changes in the stiffness are so small that the effect of the stimulation cannot be distinguished.

Subject Population

Male New Zealand White rabbits that had a body mass of 2.8 to 3.2 kilograms and that were approximately ten to twelve weeks old (these rabbits are not skeletally mature until they are approximately six months old) were obtained from a local supplier (Ace Animals, Boyertown, Pennsylvania), were examined for poor health or skeletal abnormalities, and were quarantined for six days. A total of 399 rabbits were studied in twenty experimental replicates. One replicate (replicate 5) used a total of twelve rabbits from a special Pasteurella-free colony (Skippack Animals, Denver, Pennsylvania) because our usual supplier had an inadequate inventory. This type of rabbit was not used in any other replicate in this study.

Osteotomy Procedure

Immediate preoperative preparation of each rabbit consisted of an intramuscular injection of ketamine, xylazine, acepromazine maleate, and atropine sulfate for anesthesia. The right hindlimb was prepared with a betadine solution and draped. A thirty-millimeter-long linear incision was made on the lateral aspect of the right leg, and after blunt exposure of the fibula, an osteotomy was performed with a pair of pediatric bone-cutting forceps. To ascertain that the osteotomy was complete, the proximal portion of the fibula was moved and was then re-aligned with a pair of forceps.

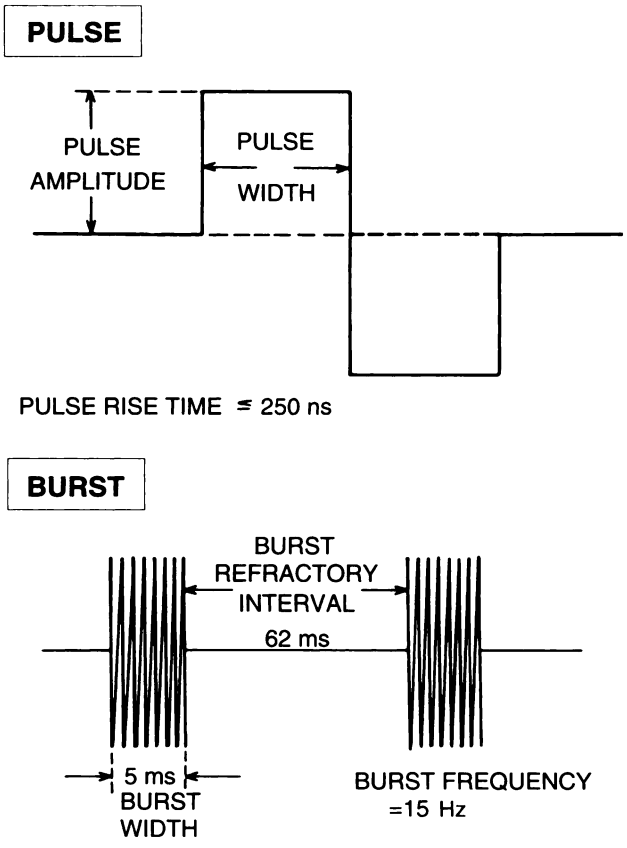

FIG. 1

Illustration of the pulse and burst characteristics of the signal that was used in the present study. Note the invariant burst frequency and burst width. Variation in the number of pulses per burst is a consequence of varying pulse width at constant burst width. The number of pulses per burst are not to scale.

TABLE I
COMPARISON OF THE STIMULUS SIGNALS

	Clinical Stimulus	50-mV, 5-μsec Stimulus
Similarities		
Burst width *(msec)*	5	5
Burst frequency *(Hz)*	15	15
Power density per pulse* *(nW/cm^3)*	1.4	1.5
Power density per burst cycle *(pW/cm^3)*	104	112
Differences		
Pulse shape	Asymmetrical	Symmetrical
Daily on-time *(hrs./day)*	8	24
Pulse width *(μsec)*	20 and 200	5
Pulse amplitude *(mV)*	17 and 135	50
Power required *(μW)*	137	30
Energy per day *(mJ)*	0.21	0.68

*Power density induced in the tissue was calculated at a radius of five millimeters and assumes a tissue conductivity of 1.4 siemens per meter (normal saline solution).

Transverse osteotomies were created within two millimeters of the middle of the shaft of the fibula in a manner that was reproducible. Cortical splitting or comminution (an *a priori* cause for rejection of an animal from the study), caused by dull cutting forceps, was usually evident at the time of the osteotomy or, if not, was detectable on the radiographs made at the conclusion of the experiment. The wound was closed with a continuous subcuticular 3-0 Vicryl (polyglactin) suture.

Aqueous procaine penicillin G (16,000 units per kilogram of body weight) was administered prophylactically to each rabbit on each of the first three days postoperatively. The rabbits were returned to their cages soon after recovery and were undisturbed until the second postoperative day. All animals had unlimited access to water and Purina Rabbit Chow (Purina Mills, Richmond, Indiana).

Strategy for Selection of the Signal

The stimulus signal is determined completely by specification of the pulse waveform, pulse amplitude, pulse width, burst width, burst frequency, and signal on-time (Fig. 1). The most efficient means for the reduction of power consumption by the signal is to reduce the pulse amplitude and the pulse width; a 50 per cent reduction in the pulse amplitude reduces consumption of electrical power by 75 per cent, and a 50 per cent reduction in the pulse width produces a 60 per cent reduction. Power savings due to the reduction of the other parameters are directly proportional to the magnitude of the reductions (for example, a 50 per cent reduction in burst width, burst frequency, or on-time produces a corresponding 50 per cent reduction in power consumption). For this reason, pulse amplitude and pulse width were the only variables chosen for this study.

Previous studies that were conducted in our laboratory provided data to support the hypotheses that asymmetry of the quasi-rectangular pulsing electromagnetic field waveform was not essential for biological efficacy and that the high-amplitude (135-millivolt), narrow (twenty-microsecond) pulse portion of the pulsing electromagnetic field that was used clinically was responsible for the therapeutic effect[32]. Therefore, the present study began by imitation of this component of the clinical signal and by systematic reduction of pulse amplitude and pulse width to determine the lower limits of efficacy.

The maximum values for pulse amplitude and pulse width that could be generated by the portable stimulation device were 200 millivolts and ten microseconds, respectively. These values were used for the first two replicates. The stimulus signals that were used for the subsequent pulse-amplitude replicates were ten, twenty-five, fifty, seventy-five, 100, and 125 millivolts, all conducted at a constant pulse width of five microseconds, and 200 millivolts, conducted at a constant pulse width of ten microseconds. The stimulus signals that were used for the pulse width experiments were one-half, one, two, three, four, five, and seven microseconds, all conducted at a constant pulse amplitude of 100 millivolts. A search coil (sixty-seven turns of number-30 AWG wire [Cooner Wire, Chatsworth, California] wound on a ten-millimeter-diameter bobbin) was used to measure pulse widths and amplitudes[4].

The values for burst width and burst frequency were the same as the values that are used clinically (Table I), for two reasons. The reductions in power that are created by variation of either are minimum, and many studies that used a fifteen-hertz burst repetition frequency component demonstrated significant biological effects[1,2,4,16,17,21,22,27,30]. Therefore, this component was unaltered because of its potential importance.

The stimulus signal bursts were applied continuously (twenty-four hours a day) because, at the time that this study was conducted, the information obtained from our laboratory suggested that "more was better"[8,38]. Other

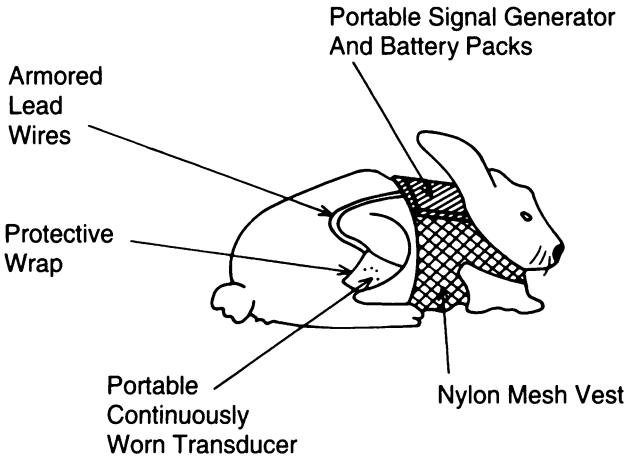

FIG. 2

Schematic illustration of the self-contained, totally portable stimulation apparatus.

TABLE II
RESULTS OF ELECTROMAGNETIC FIELD DOSIMETRY

Pulse Amplitude (*mV*)	Pulse Width (*μsec*)	Burst Width (*msec*)	Burst Frequency (*Hz*)	Peak Induced Electrical Field (*mV/cm*)	Peak Applied Magnetic Field (*μT*)	Power Density* (pW/cm^3)
Pulse-amplitude series						
10	5	5	15	0.14	28	5
25	5	5	15	0.35	71	28
50	5	5	15	0.71	141	112
75	5	5	15	1.06	212	253
100	5	5	15	1.41	283	449
125	5	5	15	1.77	353	702
200	10	5	15	2.83	1130	1796
Pulse-width series						
100	0.5	5	15	1.41	28	449
100	1	5	15	1.41	57	449
100	2	5	15	1.41	113	449
100	3	5	15	1.41	170	449
100	4	5	15	1.41	226	449
100	5	5	15	1.41	283	449
100	7	5	15	1.41	395	449

*Power density induced in the tissue was calculated at a radius of five millimeters and assumes a tissue conductivity of 1.4 siemens per meter (normal saline solution). The comparative power density of the clinical pulsing electromagnetic field is 104 picowatts per cubic centimeter.

authors[20,28,29], supported by later findings[19,30,39], have argued against this hypothesis. Although the signal was on continuously, signal-bursting meant that electrical fields were applied for only 7 per cent of the time — in other words, five milliseconds on and sixty-two milliseconds off for each sixty-seven milliseconds. Design of the lightweight portable stimulation apparatus that was used in this study was made possible by the low-power consumption of these stimulus signals.

The number of pulses per burst ranged from 250 to 2500 because the pulse width was altered in the presence of a constant (five-millisecond) burst width. Peak induced electrical fields ranged from 0.14 millivolt per centimeter (for the ten-millivolt series) to 2.83 millivolts per centimeter (for the 200-millivolt series) for the pulse amplitudes and was 1.41 millivolts per centimeter for the pulse widths. Peak applied magnetic fields ranged from twenty-eight to 1130 microtesla for the pulse amplitudes and from twenty-eight to 395 microtesla for the pulse widths (Table II).

Stimulation Apparatus

The self-contained stimulation apparatus used in this study (Fig. 2) consisted of a nylon mesh vest with two pouches (mounted dorsally) for the battery packs and one detachable pouch for the signal generator, a sealed signal-generator box, two rechargeable battery packs, a U-shaped portable inductive transducer (Fig. 3) with mu-metal electromagnetic field-shielding material on the outside and moleskin covering on the inside, and flexible armored cables to protect all of the electrical connections.

The circuitry that generated the signal and amplified the power was housed in a polymeric box that had a clear window through which a red-light-emitting diode could be seen. This diode flashed at a frequency of one hertz when the circuit was working properly, the batteries had sufficient power, and all electrical connections were intact. The active and placebo stimulator boxes appeared and functioned identically (both contained a flashing diode to indicate power consumption by the battery and proper electrical connections), except that no electrical current was applied to the transducers by the placebo boxes. The stimulator boxes were sealed and were identified alphabetically.

The inductive transducer was positioned over the site of the osteotomy and was secured to the limb with elasticized adhesive tape (Elastikon; Johnson and Johnson, New Brunswick, New Jersey). Moleskin adhesive tape was applied to the transducer to prevent or delay

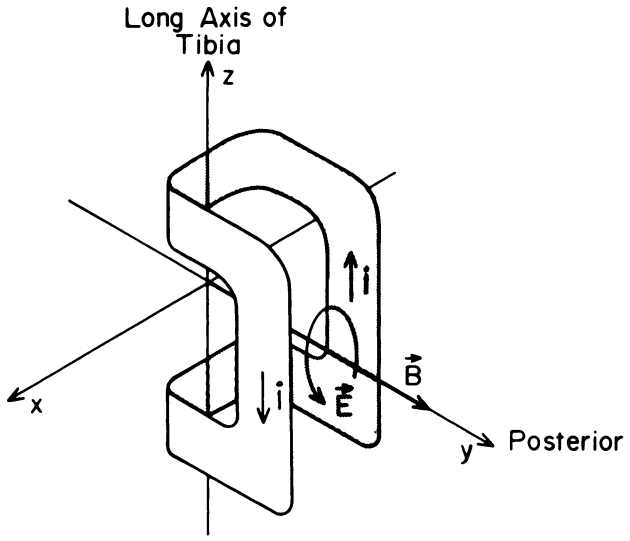

FIG. 3

Schematic illustration of the inductive transducer used to apply the desired field to the osteotomized fibula.

TABLE III

DIMENSIONLESS STIFFNESS RATIOS OF OSTEOTOMIZED TO INTACT FIBULAE ACCORDING TO PULSE AMPLITUDE*

		Stimulated Group						
Replicate	Placebo Group	10 mV	25 mV	50 mV	75 mV	100 mV	125 mV	200 mV
1	0.120 ± 0.078 (n = 6)							0.258 ± 0.192 (n = 16)
2	0.127 ± 0.044 (n = 7)							0.142 ± 0.086 (n = 9)
3	0.236 ± 0.147 (n = 7)					0.312 ± 0.175 (n = 18)		
4	0.201 ± 0.097 (n = 3)			0.464 ± 0.074† (n = 7)				
5	0.224 ± NA‡ (n = 1)			0.214 ± 0.079 (n = 5)				
6	0.225 ± 0.104 (n = 5)	0.189 ± 0.116 (n = 11)	0.113 ± 0.072 (n = 3)					
7	0.167 ± 0.076 (n = 4)		0.177 ± 0.066 (n = 8)	0.275 ± 0.098 (n = 6)	0.326 ± 0.148 (n = 6)			
8	0.287 ± 0.104 (n = 2)				0.272 ± 0.200 (n = 8)			
9	0.244 ± 0.114 (n = 4)				0.295 ± 0.139 (n = 7)	0.302 ± 0.166 (n = 5)	0.359 ± 0.237 (n = 8)	
10	0.269 ± 0.044 (n = 3)					0.144 ± 0.081§ (n = 7)		
11	0.206 ± 0.009 (n = 2)					0.216 ± 0.156 (n = 6)		
15	0.258 ± 0.164 (n = 7)					0.216 ± 0.107 (n = 10)		
16	0.242 ± 0.145 (n = 6)					0.153 ± 0.085 (n = 9)		
17	0.304 ± 0.151 (n = 5)						0.206 ± 0.133 (n = 7)	0.320 ± 0.135 (n = 6)

*Dimensionless stiffness ratios are defined as the stiffness of the osteotomized fibula (with either stimulation or placebo treatment) divided by the stiffness of the contralateral, intact, unstimulated fibula. The replicate numbers identify the order in which the experiments were conducted. All pulses were five microseconds wide, except the 200-millivolt groups of replicates 1, 2, and 17, which used ten-microsecond-wide pulses. Values are given as the mean and the standard deviation.

†$p < 0.002$.

‡NA = not applicable.

§$p < 0.05$.

destruction from gnawing. Although when the rabbits walked cyclically the transducer shifted by approximately twenty millimeters in an axial direction, the magnitude of this movement was within the region of uniform electromagnetic field. The magnetic field produced by the transducer was oriented in a medial-lateral direction. All rabbits received functionally identical, operational transducers regardless of the type of circuit box (active or placebo) that was assigned to them. Two pilot studies were conducted to develop all aspects of the experimental protocol fully. This helped to ensure that the stimulation apparatus was well tolerated by all 399 rabbits used in the study.

Blind Stimulation Protocol

Before the experiment began, a specific active or placebo stimulator box was assigned to each rabbit by an independent third party, with use of a table of random numbers arbitrarily keyed to the independently assigned housing number. The portable stimulation apparatus was applied to each rabbit on the second postoperative day. Each of the twenty experimental replicates that were performed in this study used both active and placebo stimulator boxes. The rabbits that received a placebo transducer were actually a sham experimental group; a true control group (rabbits that were not wearing any stimulation apparatus) was not used because it would have reduced the total number of rabbits available for assignment to the experimental groups. Considering our previous knowledge of this model[5,18], the historically large standard deviations of the mechanical test data obtained from it[10,24,32], the fact that the stimulation apparatus is well tolerated, and the objective of determining the efficacy of the electromagnetic stimulus signal, the use of stimulated and sham-experimental groups exclusively was thought necessary and appropriate.

The transducers were centered over the site of the osteotomy and taped (Elastikon) to the right leg of each rabbit. The unilateral design of the osteotomy, the mu-metal transducer-shielding, and a minimum one-meter separation of the rabbits from each other in large, stainless-steel cages precluded stimulation of the osteotomized fibulae that were equipped with a placebo device by stray electromagnetic fields.

TABLE IV

ABSOLUTE STIFFNESS (IN NEWTONS PER MILLIMETER) OF THE OSTEOTOMIZED FIBULAE ACCORDING TO PULSE AMPLITUDE*

		Stimulated Group						
Replicate	Placebo Group	10 mV	25 mV	50 mV	75 mV	100 mV	125 mV	200 mV
1	23.7 ± 13.1 (n = 6)							34.2 ± 19.1 (n = 16)
2	20.9 ± 9.0 (n = 6)							22.2 ± 13.5 (n = 9)
3	40.6 ± 17.2 (n = 7)					63.3 ± 34.4 (n = 18)		
4	30.4 ± 10.5 (n = 3)			55.6 ± 7.0† (n = 7)				
5	46.7 ± NA‡ (n = 1)			34.8 ± 9.8 (n = 5)				
6	39.9 ± 22.4 (n = 5)	34.0 ± 17.6 (n = 11)	21.8 ± 14.1 (n = 3)					
7	25.1 ± 19.6 (n = 4)		29.5 ± 11.3 (n = 8)	39.4 ± 14.4 (n = 6)	40.7 ± 18.9 (n = 6)			
8	32.8 ± 9.3 (n = 2)				28.2 ± 19.0 (n = 8)			
9	30.8 ± 20.0 (n = 4)				39.2 ± 20.7 (n = 7)	33.0 ± 15.8 (n = 5)	42.6 ± 36.0 (n = 8)	
10	42.0 ± 14.2 (n = 3)					26.0 ± 18.8 (n = 7)		
11	32.9 ± 9.7 (n = 2)					25.2 ± 13.3 (n = 6)		
15	40.7 ± 31.0 (n = 7)					26.1 ± 13.7 (n = 10)		
16	32.1 ± 16.2 (n = 6)					24.7 ± 13.4 (n = 9)		
17	35.8 ± 21.8 (n = 5)						26.1 ± 9.7 (n = 7)	50.5 ± 17.7 (n = 6)

*All pulses were five microseconds wide, except the 200-millivolt groups of replicates 1, 2 and 17, which used ten-microsecond-wide pulses. Values are given as the mean and the standard deviation.

†$p < 0.01$.

‡NA = not applicable.

The rabbits were allowed free activity in individual cages and unlimited access to water and food (Purina Rabbit Chow). Qualitative evaluations indicated that the activity levels of the rabbits were approximately equivalent to each other and virtually identical to the preoperative levels. All rabbits wore the equipment continually for fourteen days, and pulse burst electromagnetic stimulation was applied continuously to the rabbits who had the active-stimulation apparatus.

The health of each rabbit, the adequacy of the food and housing conditions, and the performance of the equipment were checked daily. Stimulator boxes that malfunctioned were replaced immediately without unblinding of the experiment: a sealed envelope assigned to each stimulator contained alphabetical identification of two spare stimulator boxes of identical function. Body mass was a reasonably good indicator of health and was measured at the beginning and the end of the study, and every three days during the study.

Assessment of Healing

All of the rabbits were killed on the sixteenth postoperative day by an intravenous injection of 1.5 milliliters of T-61 euthanasia solution (sodium pentobarbital). Both the right, osteotomized fibula and the left, intact fibula of each rabbit were immediately excised and immersed in Hanks buffered salt solution. Each osteotomized fibula was radiographed in the anterior-posterior and medial-lateral planes.

Each fibula (osteotomized and intact) was individually placed perpendicularly across the two lower horizontal supports of a rigid custom-machined three-point-bending test fixture. A linear voltage displacement transducer was fastened to the lower section of this fixture, with the spring-loaded plunger of the transducer touching the upper section of the fixture; thus, relative motion of the upper and lower supports was linearly proportional to the specimen strain as measured by the voltage output of the transducer. The osteotomized sample was carefully positioned in the longitudinal direction so that the center (upper) support of the fixture touched the center of the fracture callus. The intact contralateral fibula was placed in the same longitudinal position. No preload was applied before testing. All fibulae were loaded within their elastic range at a relative support displacement rate of four millimeters per minute on a uniaxial servohydraulic materials-testing system (CGS, Minneapolis, Minnesota). The

TABLE V

ABSOLUTE STIFFNESS (IN NEWTONS PER MILLIMETER) OF THE INTACT, UNSTIMULATED, CONTRALATERAL FIBULAE ACCORDING TO PULSE AMPLITUDE*

Replicate	Placebo Group	Stimulated Group						
		10 mV	25 mV	50 mV	75 mV	100 mV	125 mV	200 mV
1	208.8 ± 27.1 (n = 6)							154.6 ± 47.4† (n = 16)
2	163.7 ± 31.2 (n = 6)							165.7 ± 53.3 (n = 9)
3	191.1 ± 43.9 (n = 7)					212.4 ± 87.4 (n = 18)		
4	158.6 ± 36.1 (n = 3)			121.0 ± 14.1‡ (n = 7)				
5	208.3 ± NA§ (n = 1)			166.9 ± 22.7 (n = 5)				
6	171.2 ± 34.0 (n = 5)	189.2 ± 44.7 (n = 11)	189.2 ± 9.3 (n = 3)					
7	140.3 ± 48.1 (n = 4)		167.9 ± 27.8‡ (n = 8)	143.6 ± 18.3 (n = 6)	125.8 ± 20.3 (n = 6)			
8	115.9 ± 9.4 (n = 2)				113.9 ± 30.9 (n = 8)			
9	119.6 ± 31.9 (n = 4)				135.9 ± 38.5 (n = 7)	115.1 ± 16.9 (n = 5)	112.1 ± 28.5 (n = 8)	
10	153.3 ± 31.4 (n = 3)					178.3 ± 39.7 (n = 7)		
11	158.4 ± 39.9 (n = 2)					127.0 ± 39.8 (n = 6)		
15	160.1 ± 32.0 (n = 7)					125.6 ± 35.7 (n = 10)		
16	139.0 ± 17.3 (n = 6)					162.2 ± 41.0 (n = 9)		
17	116.8 ± 38.0 (n = 5)						143.3 ± 56.3 (n = 7)	161.7 ± 18.0 (n = 6)

*All pulses were five microseconds wide, except the 200-millivolt groups of replicates 1, 2 and 17, which used ten-microsecond-wide pulses. Values are given as the mean and the standard deviation.

†$p < 0.02$.

‡$p < 0.05$. In replicate 7, only the twenty-five-millivolt group was significantly different from the seventy-five-millivolt group.

§NA = not applicable.

axial load applied to the bending fixture was measured with a 220-newton-capacity load-cell and recorded on the y axis of an x-y chart recorder. The deflection of the three-point bending-jig was recorded on the x axis of the chart-recorder[32].

Mechanical testing was halted before the yield point to preserve the integrity of the fracture callus. Bending stiffness (the slope of the load-deformation curve) was the only mechanical property measured. Mechanical testing was completed within ten minutes after the cessation of circulation of each rabbit.

Immediately after the non-disruptive mechanical testing, samples were sectioned longitudinally with a low-speed diamond-blade saw that was partially immersed in 10 per cent neutral buffered formalin. All samples were then fixed in the formalin solution for later histological processing.

Analysis of the Data

The three-point bending stiffness of each fibula was determined with use of a digitizing tablet to measure the maximum slope of the linear elastic portion of the load-deformation curve. The stiffness determination of each test was confirmed by sighting along the tracing and by optical confirmation of the calculated slope. This verification was performed by an unbiased, independent observer who was skilled in these measurements and, as with all of the other participants in the experiment, who was blinded with regard to the identity of the specimen. The average error introduced by the second independent stiffness determination was 0.35 per cent.

To minimize inter-animal variations, the stiffness of each osteotomized fibula was divided (normalized) by the stiffness of the intact, contralateral fibula (Tables III and VI). This quotient, the stiffness ratio, was considered the most important and relevant parameter. Absolute stiffness values of the left and right fibulae were also measured and analyzed. Only those absolute values that provide important information about the corresponding stiffness-ratio data are reported (Tables IV, V, VII, and VIII).

Changes in the weight of the animals were determined from measurements made on the second and sixteenth postoperative days (the beginning and end of the stimulation interval). All data from any particular rabbit were excluded from consideration if that rabbit

TABLE VI

DIMENSIONLESS STIFFNESS RATIOS OF THE OSTEOTOMIZED TO THE INTACT FIBULAE ACCORDING TO PULSE WIDTH*

Replicate	Placebo Group	Stimulated Group						
		0.5 μsec	1 μsec	2 μsec	3 μsec	4 μsec	5 μsec	7 μsec
11	0.206 ± 0.009 (n = 2)	0.190 ± 0.101 (n = 8)		0.240 ± 0.100 (n = 5)			0.216 ± 0.156 (n = 6)	
12	0.156 ± 0.029 (n = 5)			0.265 ± 0.204 (n = 9)		0.211 ± 0.107 (n = 8)		
13	0.155 ± 0.081 (n = 6)		0.130 ± 0.074 (n = 8)		0.147 ± 0.078 (n = 8)			
14	0.156 ± 0.074 (n = 6)							0.302 ± 0.114† (n = 7)
16	0.242 ± 0.145 (n = 6)				0.267 ± 0.175 (n = 8)		0.153 ± 0.085 (n = 9)	
18	0.279 ± 0.150 (n = 3)				0.211 ± 0.082 (n = 9)			0.323 ± 0.146 (n = 8)
19	0.192 ± 0.160 (n = 7)							0.200 ± 0.113 (n = 6)
20	0.227 ± 0.162 (n = 6)							0.204 ± 0.146 (n = 8)

*All pulses were of 100-millivolt amplitude. The five-microsecond, 100-millivolt data and corresponding placebo data from replicates 3, 9, 10, 11, 15, and 16 (Table III) were also part of the pulse-width series but were not repeated in this table. Values are given as the mean and the standard deviation.

†$p < 0.05$.

exhibited a change in body weight (calculated as described previously) of 15 per cent or more, had a cortical malalignment of more than 50 per cent in both the anterior-posterior and the medial-lateral planes (as determined from the radiographs), had either a comminuted osteotomy site or split cortices, or had lost two or more days of stimulation.

Each experimental replicate was unblinded and the rabbits were identified as having received either an active or a placebo stimulator only after each animal had been included in, or had been rejected from, analysis (for the reasons just mentioned) and after all radiographic and mechanical testing data had been recorded in the laboratory notebook.

The mean stiffness of the intact fibula, the osteotomized fibula, and the stiffness ratio (the value for the osteotomized fibula divided by that for the intact fibula) were calculated. Differences in group means (active stimulators compared with placebo stimulators) were attributed to chance fluctuations (null hypothesis) or to the specific electromagnetic stimulus used (alternative hypothesis). Similar (multiple) groups were compared with use of a one-factor one-way analysis of variance, and the Scheffé F test was used for *post hoc* comparisons with use of Statview-II software (Abacus Concepts, Berkeley, California).

Results

Response of the Animals

The vest-mounted apparatus and the experimental protocol were well tolerated by the rabbits. Each transducer needed to be rewrapped at least once, and an average of twice, during the course of the study. Weight loss was minimum (mean, 2.4 per cent) and was not correlated with any experimental replicate or stimulus signal group.

Fifty of the original 399 animals were rejected from the study before unblinding and analysis because of a comminuted fracture (fourteen), excessive weight change (twelve), death before the study period ended (twelve [six of which were the special Pasteurella-free rabbits that were used in replicate 5]), improper alignment of the fracture (six), or some other reason (six). These rejection criteria were established *a priori*. Except for replicate 5, the rejections were distributed throughout the study and did not correlate with specific signal parameters, active or placebo stimulator status, or experimental replicate. The variable occurrence of these rejections, in addition to the rabbit supply and the animal-housing limitations, are the reasons why seemingly similar experimental replicates had varying sample sizes.

There were no adverse tissue effects attributable to the stimulation.

Data on the Placebo-Stimulator Group

The ratio of the stiffness of the osteotomized fibula to that of the intact, contralateral fibula for all animals equipped with a placebo device ranged from 0.120 ± 0.078 to 0.304 ± 0.151 (Table III). The mean stiffness ratio of the placebo groups in replicates 1 and 2 was 0.124, which is consistent with the data of others who have used this model with similar sample sizes[10]. Although the mean stiffness ratios for the placebo groups in replicates 3 through 20 were larger, none of the twenty ratios were significantly different. The only significant difference in all possible comparisons of the absolute and relative stiffness of the placebo group was

TABLE VII

ABSOLUTE STIFFNESS (IN NEWTONS PER MILLIMETER) OF THE OSTEOTOMIZED FIBULAE ACCORDING TO PULSE WIDTH*

Replicate	Placebo Group	Stimulated Group						
		0.5 μsec	1 μsec	2 μsec	3 μsec	4 μsec	5 μsec	7 μsec
11	32.9 ± 9.7 (n = 2)	22.8 ± 15.6 (n = 8)		27.8 ± 18.7 (n = 5)			25.2 ± 13.3 (n = 6)	
12	21.2 ± 3.3 (n = 5)			31.1 ± 16.5 (n = 9)		33.1 ± 18.6 (n = 8)		
13	22.4 ± 10.7 (n = 6)		21.5 ± 9.5 (n = 8)		26.0 ± 16.6 (n = 8)			
14	20.2 ± 8.9 (n = 6)							38.1 ± 9.8† (n = 7)
16	32.1 ± 16.2 (n = 6)				39.4 ± 26.4 (n = 8)		24.7 ± 13.4 (n = 9)	
18	19.5 ± 12.8 (n = 3)				33.0 ± 12.9 (n = 9)			44.1 ± 17.9 (n = 8)
19	22.8 ± 15.2 (n = 7)							32.8 ± 15.5 (n = 6)
20	37.4 ± 25.4 (n = 6)							25.8 ± 13.3 (n = 8)

*All pulses were of 100-millivolt amplitude. The five-microsecond, 100-millivolt data and corresponding placebo data from replicates 3, 9, 10, 11, 15, and 16 (Table III) were also part of the pulse-width series but were not repeated in this table. Values are given as the mean and the standard deviation.

†$p < 0.05$.

that the intact fibulae from replicate 1 (mean, 208.8 ± 27.1) were stiffer ($p < 0.05$) than the intact fibulae from replicate 18 (mean, 68.4 ± 19.5) (Tables V and VIII).

Pulse-Amplitude Data

A dose-response relationship was discovered for absolute and relative fibular stiffnesses as a function of varying pulse amplitudes at a constant pulse width of five microseconds (Tables III, IV, and V). The ten and twenty-five-millivolt-amplitude groups (replicates 6 and 7, Table III) showed no increase of the stiffness, and these two signals were not studied further.

The fifty-millivolt-amplitude signal was studied in three separate replicates (4, 5, and 7) (Table III). Replicate 4 showed a highly significant ($p < 0.002$) increase of the stiffness ratio (0.464 ± 0.074 compared with 0.201 ± 0.097 in the placebo group). Although the stiffness ratios for the stimulated fibulae in this replicate were larger because of significantly ($p < 0.05$) weaker contralateral fibulae (121.0 ± 14.1 compared with 158.6 ± 36.1 newtons per millimeter) (Table V), the significant ($p < 0.01$) increase of the absolute stiffnesses (55.6 ± 7.0 compared with 30.4 ± 10.5 newtons per millimeter) (Table IV) of the stimulated fibulae strongly supports the efficacy of the fifty-millivolt signal in this replicate. Furthermore, the seven stimulated fibulae in this replicate were so distinctly different from the three placebo-treated fibulae that visual comparison of the gross specimens and of the radiographic images of these fibulae enabled clear differentiation of the stimulated and the placebo-treated specimens.

Illness, and the subsequent 50 per cent rate of mortality, of the special Pasteurella-free colony of rabbits used in the second fifty-millivolt experiment (replicate 5) may be partially responsible for the lack of a significant difference between the mean fibular stiffnesses of the stimulated and the placebo groups.

The absolute stiffness (39.4 ± 14.4 newtons per millimeter) (Table IV) and the relative stiffness of the osteotomized fibulae that were treated with the fifty-millivolt signal were larger, but not significantly so, than the absolute stiffness (25.1 ± 19.6 newtons per millimeter) and the relative stiffness of the fibulae that had a placebo device in replicate 7. In this replicate, the stiffnesses of the intact fibulae in the active and placebo groups (143.6 ± 18.3 and 140.3 ± 48.1 newtons per millimeter, respectively) (Table V) were virtually identical.

The seventy-five-millivolt pulse-amplitude stimulus increased the mean stiffness ratio (0.326 ± 0.148 compared with 0.167 ± 0.076) in one (replicate 7) of the three replicates (7, 8, and 9) in which it was used, but it is important to note that this increase was detected only with use of the Fisher protected least-significant-difference test ($p < 0.05$), the least conservative test (the most likely to show an erroneous difference). The Scheffé F test, which was used for all other *post hoc* comparison tests in this study, is the most conservative. The absolute stiffness of the osteotomized fibulae in the seventy-five-millivolt group was larger than the absolute stiffness in the corresponding placebo group (40.7 ± 18.9 compared with 25.1 ± 19.6 newtons per millimeter) (Table IV), but it was not significantly different.

The stimulus signal with a 100-millivolt pulse amplitude and a five-microsecond pulse width failed to increase the stiffness in any experiment significantly. One replicate (10) actually showed a significant ($p < 0.05$) decrease in the stiffness ratio (Table III). However, differences in this ratio were aided by slightly stiffer intact,

TABLE VIII

ABSOLUTE STIFFNESS (IN NEWTONS PER MILLIMETER) OF THE INTACT, UNSTIMULATED, CONTRALATERAL FIBULAE ACCORDING TO PULSE WIDTH*

Replicate	Placebo Group	Stimulated Group						
		0.5 μsec	1 μsec	2 μsec	3 μsec	4 μsec	5 μsec	7 μsec
11	158.4 ± 39.9 (n = 2)	120.2 ± 46.3 (n = 8)		119.4 ± 52.0 (n = 5)			127.1 ± 39.7 (n = 6)	
12	138.0 ± 20.7 (n = 5)			129.8 ± 38.3 (n = 9)		150.9 ± 39.6 (n = 8)		
13	151.2 ± 26.0 (n = 6)		180.1 ± 44.5 (n = 8)		177.8 ± 62.6 (n = 8)			
14	133.2 ± 24.1 (n = 6)							130.9 ± 19.5 (n = 7)
16	139.0 ± 17.3 (n = 6)				152.2 ± 38.8 (n = 8)		162.2 ± 41.1 (n = 9)	
18	68.4 ± 19.5 (n = 3)				160.5 ± 36.8† (n = 9)			145.6 ± 38.6‡ (n = 8)
19	135.3 ± 34.7 (n = 7)							179.2 ± 67.1 (n = 6)
20	176.3 ± 48.3 (n = 6)							137.7 ± 23.5 (n = 8)

*All pulses were of 100-millivolt amplitude. Values are given as the mean and the standard deviation.
†$p < 0.01$.
‡$p < 0.05$.

contralateral fibulae in the stimulated group (178.3 ± 39.7 compared with 153.3 ± 31.4 newtons per millimeter) (Table V).

A non-significant trend toward an increase in both the relative and the absolute stiffness was observed in one (replicate 9) of the two 125-millivolt-signal replicates. The osteotomized fibulae in the 200-millivolt group in replicate 17 had an increased absolute stiffness (50.5 ± 17.7 compared with 35.8 ± 21.8 newtons per millimeter) (Table IV) but a low comparative stiffness ratio (0.320 ± 0.135 compared with 0.304 ± 0.151) (Table III) because the intact, contralateral fibulae were weaker in the placebo-treated animals than in the stimulated animals (116.8 ± 38.0 and 161.7 ± 18.0 newtons per millimeter, respectively) (Table V).

Pulse-Width Data

A dose-response relationship was discovered for absolute and relative fibular stiffnesses as a function of varying pulse width at a constant pulse amplitude of 100 millivolts (Tables VI, VII, and VIII [although not reported in the tables, the 100-millivolt, five-microsecond data obtained in replicates 3, 9, 10, 15, and 16 were also included in the analysis of the pulse-width series]).

The relative stiffness ratio was significantly increased (0.302 ± 0.114 compared with 0.156 ± 0.074) in one (replicate 14) of the four replicates conducted with the use of the seven-microsecond pulse-width signal (Table VI). The absolute stiffnesses of the stimulated osteotomized fibulae in this replicate were also significantly ($p < 0.05$) stiffer than the absolute stiffnesses in the corresponding placebo group (38.1 ± 9.8 compared with 20.2 ± 8.9 newtons per millimeter) (Table VII), and the stiffnesses of the intact, contralateral fibulae were virtually identical in the two groups (130.9 ± 19.5 compared with 133.2 ± 24.1 newtons per millimeter) (Table VIII).

Discussion

Power Considerations

The principal result of this study was the identification, with use of a blinded protocol, of a range of specific pulse amplitudes and pulse widths of an electromagnetic stimulus signal waveform that can significantly increase the stiffness of the fracture callus of an established rabbit model. This finding is important because the electrical power consumed by the most efficient of these signals is 78 per cent less than that consumed by the pulsing electromagnetic field that is now used clinically. Minimum consumption of electrical power enables the development of small, lightweight, totally cast-incorporated, disposable clinical devices that will promote patient compliance and will ensure administration of the prescribed stimulus dosage. Ease and economy of use of such devices will also encourage double-blind studies, which are regarded as essential for the documentation of clinical efficacy[36].

It must be noted that, although the fifty-millivolt, five-microsecond signal requires approximately one-fifth of the electrical power as the pulsing electromagnetic field used clinically, the calculated electromagnetic power density available to the exposed tissue is nearly identical for the two fields (Table I). It is also important that neither the signal that is used clinically nor any of the signals used in this study contain sufficient energy to heat the exposed tissue appreciably.

Specific Signal Parameters

The selection of specific signal parameters relies primarily on experimental verification, since the mechanism by which electromagnetic stimulation affects cells

is not presently understood. Order-of-magnitude choices for signal amplitudes are guided by electrical field amplitudes measured from stress-generated-potential experiments. Calculated peak electrical-field amplitudes of 0.71 to 1.41 millivolts per centimeter are induced in the tissue by stimulus signal amplitudes of fifty to 100 millivolts (Table II) and are comparable with the electrical field amplitudes of one millivolt per centimeter measured *in vitro* in stressed wet bone[3,23,25,31]. The data obtained in this study are consistent with the hypothesis that stress-generated electrical fields mediate osseous growth, repair, and remodeling by an electrically mediated Wolff law-type feedback mechanism and support the claim that electromagnetic stimulation functions as an imitator of the endogenous electrogenerative properties of living bone.

It is important to note that the signal that is perceived by the cell, not by the field-sensing search coil, is what is biologically relevant. Field amplitudes that are measured with the search-coil probe may not be equivalent to the field amplitudes experienced by the cell membrane. The specificity of cellular response to different variations in signal parameter, shown by this and other studies[4,12,19,27,32,37,39], emphasizes the need for an understanding of the specific signal dose that is applied to the target cells. Calculation of the field amplitudes on the cellular level, similar to that performed for other stimulation modalities[11], will enable proper comparison of induced fields in experiments that use different stimulation modalities and different animal models.

The signal frequencies that were used in the present study are consistent with the frequencies that were found to be effective in other studies[10,27]. Although the present study used symmetrical rectangular waves, Fourier transforms of these pulse widths show that the fundamental frequencies (first harmonic) of the five and seven-microsecond pulse-width group are approximately 100 and seventy-one kilohertz, respectively. These values are comparable with the single sinusoidal sixty-kilohertz frequency used in capacitive coupling[10]. The clinical asymmetrical pulsing electromagnetic field waveform also has higher-order harmonics in this range of frequencies (sixty to 100 kilohertz).

Pulse bursts used in the present study were repeated at fifteen hertz. Many other studies have shown marked biological effects when this frequency component was used for bursts[4,19,30] or for continuous sinusoidal frequencies[16,27]. The fifteen-hertz component has also been reported to be important in the response of bone to dynamic loading[14]. Although not every stimulus that has a fifteen-hertz component produces significant biological effects[13], perhaps wide variations in pulse amplitude and pulse width are effective when applied in conjunction with a fifteen-hertz burst repetition frequency.

Dose-response studies of burst width, burst frequency, wave shape, and duty cycle are needed but have not been fully explored, nor have other ranges of pulse amplitude and pulse width[26], as far as we know. There may be other ranges of signal amplitude and pulse width that would be equally effective in this model.

Sources of Variability in the Data

Technique for Application of the Transducer

A potential explanation for the increase in the mean stiffnesses in the placebo group (in replicates 1 and 2 compared with 3 through 20) was found from careful review of the experimental protocol. During approximately the first five of these replicates, the elasticized adhesive tape that was used to secure the electromagnetic transducer to the osteotomized limb of the rabbit was applied with more tension than was necessary. This often caused an edematous foot and required rewrapping of the transducer with the use of less tension. The technical staff subsequently became sufficiently skilled in transducer-wrapping, and edema became uncommon. Thus, a learning curve for the application of the transducer, not for the operative technique (the same one of us [D. P.] performed approximately 150 fibular osteotomies during the course of a previous study[32]), may be responsible for the trend of increasing mean stiffnesses in the placebo group.

The learning curve for application of the transducer and the inability to reproduce the results seen in the early experimental replicates may be related according to the following hypothesis. If it is assumed that electromagnetic stimulation can benefit only non-optimally healing tissues, then the stiffness of only non-optimally healing calluses can be increased by specific electromagnetic stimulation. Excessive tension in the tape used in wrapping may have induced a subnormal healing environment, which then became responsive to specific electromagnetic stimuli. When the wrapping technique improved and less tension was applied to the adhesive tape, a return to a normal (near-optimum) healing environment increased the stiffness of the placebo-treated fibulae, as shown by the higher mean ratio of control stiffness, and reduced the amount by which an electromagnetic stimulus could increase the fibular stiffness. A reduction in the difference in means in a model in which the standard deviations are typically half of the mean substantially reduces the ability to distinguish true differences in sample means statistically.

If this hypothesis is true, then specific electromagnetic stimuli may be useful in clinical applications whenever a subnormal tissue-repair, regeneration, or maintenance process recurs, as in delayed union or fractures with extensive soft-tissue damage, impaired blood supply, or other factors known to impede normal healing.

Mechanical Testing

The three-point-bending measurements of fibular callus stiffness may have been partially responsible for the large standard deviations (50 to 60 per cent of the

mean) and subsequent difficulty in the resolution of true differences in sample means. Two-plane radiographic examination of the osteotomized fibulae usually showed partial calcification at various anatomical locations in the sixteen-day-old fracture callus. This varying location of calcification (even of the same amount) can cause large differences in the three-point-bending stiffness, as explained with the simple beam theory. This is supported by the observation that the standard deviations of the intact, contralateral fibulae were typically, at most, one-quarter of the mean.

Tensile mechanical testing of sixteen-day-old osteotomized rabbit fibulae showed comparable stiffnesses but smaller standard deviations[5]. Smaller standard deviations were also observed in torsional testing of rabbit fibulae[33]. Tensile and, to a slightly less extent, torsional mechanical testing of whole-bone specimens are less sensitive to varying locations of the same amount and type of callus material. Three-point bending offers ease and speed of excision and testing, minimum disruption of the callus during mounting of the specimen in the test fixture, and simplicity of alignment. It also obviates the need to drill, pin, pot, or accurately align the specimen in the test machine. However, when differences in group means are small, the smaller variability associated with tensile testing makes it the preferable mechanical test for future assessments of fibular callus stiffness in rabbits.

In conclusion, a new range of specific electromagnetic signals that can stimulate healing in the rabbit fibular osteotomy model has been identified. Given the historical usefulness of this model in the forecast of clinical effectiveness of electrical stimulation therapies, the signals that were shown to be effective in the present study offer an excellent potential for application in humans. Low-power consumption of these specific waveform parameters enables a new generation of clinical devices that have many logistical benefits for the patient and the physician.

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