Pulsed Magnetic Fields Accelerate Cutaneous Wound Healing in Rats

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Background: Previous studies of pulsed magnetic fields have reported enhanced fracture and chronic wound healing, endothelial cell growth, and angiogenesis. This study characterizes the biomechanical changes that occur when standard cutaneous wounds are exposed to radiofrequency pulsed magnetic fields with specific dosage parameters, in an attempt to determine whether return to functional tensile strength could be accelerated in wound healing.
Methods: There were two study phases and a total of 100 rats. In phase 1, wounds were exposed to a 1.0-G pulsed magnetic field signal in clinical use for wound repair for 30 minutes twice daily for 21 or 60 days. Phase 2 was a prospective, placebo-controlled, double-blind trial in which rats were treated for 30 minutes twice daily with three different low-amplitude signals (0.02 to 0.05 G), configured assuming a Ca^{2+} binding transduction pathway, for 21 days. A midline, 8-cm, linear skin incision was made on the rat dorsum. Tensile strength was determined by measuring the point of rupture of the wound on a standard tensiometer loaded at 0.45 mm/second.
Results: The mean tensile strength of treated groups in phase 1 was 48 percent ($p < 0.001$) greater than that of controls at 21 days; there was no significant difference at 60 days. In phase 2, the treated groups showed 18 percent (not significant), 44 percent, and 59 percent ($p < 0.001$) increases in tensile strength over controls at 21 days.
Conclusion: The authors successfully demonstrated that exposing wounds to pulsed magnetic fields of very specific configurations accelerated early wound healing in this animal model, as evidenced by significantly increased wound tensile strength at 21 days after wounding. (*Plast. Reconstr. Surg.* 120: 425, 2007.)

Pulsed magnetic fields have been shown to enhance healing of delayed and nonunion fractures, fresh fractures, and spine fusions.[1–6] Additional clinical indications have been reported in double-blind studies for the treatment of avascular necrosis,[7,8] tendinitis,[9] and osteoarthritis.[10] Radiofrequency pulsed magnetic fields, of the type utilized in this study, have been shown in double-blind clinical studies to enhance chronic wound repair[11,12] and to reduce pain and edema in acute ankle sprains[13,14] and acute whiplash injuries.[15,16] In addition, their signals have been reported to enhance skin microvascular blood flow in both healthy[17] and diabetic[18] individuals. Pulsed magnetic fields significantly reduced or healed venous ulcers in a randomized control study.[19] A meta-analysis[20] of randomized clinical trials using pulsed magnetic fields on soft tissues and joints showed that they were effective in accelerating the healing of skin wounds.[21,22] Pulsed magnetic field signals have been successfully used to treat chronic pain associated with connective tissue (cartilage, tendon, ligaments, and bone) injury and joint-associated soft-tissue injury.[23,24] Studies have also demonstrated that they promote endothelial cell growth in vitro.[25] In a similar study, they increased the degree of endothelial cell tubulization and proliferation three-fold.[26]

Recently, our laboratory showed that pulsed magnetic fields stimulate neovascularization when they are applied to a transplanted tail ar-

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terial loop in a rat groin model.[27] This was the first time that they were shown to promote angiogenesis in an in vivo model. More recently, this study was extended to demonstrate that the angiogenesis produced in this same model was sufficient to allow elevation of a free flap based on the newly produced vascular bed.[28]

The objective of this study was two-fold: first, to identify, in a controlled manner, a pulsed magnetic field radiofrequency signal that would provide significantly increased tensile strength to a linear wound at 3 weeks after wounding; and second, to identify a lower-amplitude signal that could produce the same effect (i.e., more rapid tensile strength increase) in the same period of time.

MATERIALS AND METHODS

The rat wound model utilized in this study has been well characterized both biomechanically and biochemically.[29] Healthy, young adult (≥300 g), male Sprague-Dawley rats were used. The study was approved by the Institutional Animal Care and Use Committee of Montefiore Medical Center and abided by all the requirements of the Animal Welfare Act.

The animals were anesthetized with an intraperitoneal dose of ketamine 75 mg/kg and medetomidine 0.5 mg/kg. After adequate anesthesia had been achieved, the dorsum was shaved, prepared with a diluted Betadine/alcohol solution, and draped using a sterile technique. With a no. 10 scalpel, an 8-cm linear incision was made through the skin down to the fascia on the dorsum of each rat. The wound edges were bluntly dissected to break any remaining dermal fibers, leaving an open wound approximately 4 cm wide at the widest point of separation. Hemostasis was obtained with applied pressure to avoid any damage to the skin edges. The skin edges were then closed with a 4-0 Ethilon running suture. Postoperatively, the animals received buprenorphine 0.1 to 0.5 mg/kg intraperitoneally. They were placed in individual cages and received food and water ad libitum.

In phase 1 of the study, 40 Sprague-Dawley rats were divided into four groups of 10 animals each (groups 1A, 1B, 1C, and 1D). The active groups were treated with signal I. Group 1A received a high-amplitude (1.0-G) pulsed magnetic field signal (signal I), for 30 minutes, twice a day, for 21 days. Group 1B did not receive a signal and served as the control group. Group 1C received pulsed magnetic field signal I for 30 minutes, twice a day, for 60 days. Group 1D did not receive a signal and was the control group for the 60-day time point.

Phase 2 of the study was carried out in a randomized, double-blind manner utilizing three low-amplitude pulsed magnetic field signals (signal II, III, and IV). Sixty Sprague-Dawley rats were divided into three active and three corresponding sham groups of 10 animals each (groups 2A and 2B, 2C and 2D, and 2E and 2F). Group 2A received pulsed magnetic field signal II, group 2C received signal III, and group 2E received signal IV. Groups 2B, 2D, and 2F received a sham signal and served as the control groups for their respective signals. Both active and sham animals were treated for 30 minutes, twice a day, for 21 days.

In total, four radiofrequency pulsed magnetic field signals were used in the study. Signal I, used in phase 1, consisted of a 65-μsec burst of 27.12-MHz sinusoidal waves inducing a 1-G high-amplitude peak magnetic field in the tissue, repeating at 600 per second. This signal is used routinely to treat chronic wounds.[20] The three phase 2 signals were configured as follows: signal II was a 1-msec burst of 27.12 MHz repeating at five bursts per second, 0.02 G of peak amplitude; signal III was identical to signal II, but at 0.05 G of peak amplitude; and signal IV was a 2-msec burst of 27.12 MHz repeating at five bursts per second, with a peak amplitude of 0.05 G (SofPulse; Ivivi Technologies, Inc., Northvale, N.J.). All phase 2 signals were configured a priori to induce a wideband time-varying magnetic field, and corresponding electric field, in the tissue containing frequency components at an amplitude sufficient to modulate biochemical cascades relevant to tissue repair.[30,31]

In phase 1, animals were positioned individually directly on a 9-inch coil applicator; they were confined to ensure that treated animals received signal I with a consistent 1 ± 0.1-G signal dose at the incision site. Phase 1 control animals were treated in an identical manner. In phase 2, five animals were placed in a standard plastic rat cage, with all metal portions removed, and allowed to roam freely. The cage was placed within a 14 × 21-inch electrical coil that was positioned horizontally and at 3.5 inches in height versus the cage floor. Each signal amplitude was checked throughout the study with an NIST traceable calibrated field probe (model FCC-301-1-MR1; Fischer Custom Communications, Torrance, Calif.) connected to a calibrated 100-MHz oscilloscope (model 2358; Tektronix, Beaverton, Ore.). The signal amplitude did not vary by more than ±10 percent for all active groups. The field probe allowed waveform consis-

tency to be verified throughout the study. There were two cages each for all control and active groups, allowing 10 animals to be treated per group. Each cage had an individually coded signal generator.

After 21 days, all animals in each phase were anesthetized, as described previously, and euthanized. The backs of the animals were shaved, and the skin, including the linear wound, was excised. The same procedure was performed for groups 1C and 1D phase 1 animals at 60 days.

Tensile Strength Determination

For all animals in both stages of the study, tensile strength was evaluated immediately after wound excision. Two 1-cm-wide strips of skin were transected (perpendicular to the scar) from each animal and used to measure tensile strength (expressed in kg/cm^2). Each sample was mounted in a special clamp that tightly held each end. The sample was positioned so that the incision was at the midline between the clamped edges. The clamp was mounted on a tensiometer (model 922MTC; Comten, St. Petersburg, Fla.), and load was applied at 0.45 mm/second using a 20-lb load cell. The maximum force generated at wound failure was recorded. Final tensile strength for comparison was determined by taking the mean of the maximum load (in kg/cm^2) of all strips from all wounds.

Statistical Analysis

Mean tensile strength was compared for each group. Data were analyzed using SigmaStat 3.0 software (SPSS, Chicago, Ill.). All data passed the Kolmogorov-Smirnov normality test, which allowed parametric statistical analyses to be used. One-way analysis of variance was used for all comparisons. Significance was accepted at $p \leq 0.05$.

RESULTS

Tensile strength was calculated as the maximum breaking strength (in kilograms) per cross-sectional area (in square centimeters). The results (expressed in kg/cm^2 ± SD) are shown in Table 1. Wounds treated with the 65-μsec clinical signal (signal I) had a mean breaking strength that was 48 percent higher than that of controls at 21 days ($p < 0.001$). Wounds treated with signal I were 2 percent stronger than control wounds at 60 days, which was not statistically significant. The mean tensile strength for phase II treated wounds was 18 percent ($p = 0.126$), 44 percent ($p < 0.001$), and 59 percent ($p < 0.001$) higher than that of the corresponding control groups for signals II, III, and IV, respectively. Signal II, which induced the lowest amplitude to the wound target (0.02 G) had no significant effect. In contrast, signal IV, which had the longest burst duration (2 msec versus 1 msec) and higher amplitude (0.05 G) had the most significant effect on wound repair in this model. The 21-day results are summarized as the ratio of mean tensile strength ± SD of active to corresponding control group in Figure 1.

DISCUSSION

Noninvasive, nonthermal pulsed magnetic fields are successful therapies for healing nonunion fractures, for palliative relief of pain and edema, and for healing chronic wounds. The radiofrequency devices used in this study have been cleared by the U.S. Food and Drug Administration for pain and edema relief, and a governmental decision memo[32] has determined that they are effective in the off-label application of healing chronic wounds, such as pressure sores and diabetic leg and foot ulcers. Our study is the first to present objective quantitative data on the effect of radiofrequency pulsed magnetic fields on biomechanical healing rates in an animal linear wound model.

The exact mechanism of action of the pulsed magnetic field signal on cutaneous wound healing has not yet been completely identified. Certainly, at the cellular level, pulsed magnetic fields have been shown to enhance production of transform-

Table 1. Tensile Strength of Pulsed Magnetic Field–Treated and Sham Wounds*

	Tensile Strength (kg/cm² ± SD)		
PMF Signal	Treated	Sham	ANOVA (p)
I: 65 μsec, 600 Hz, 1 G	35.7 ± 9.1	24.1 ± 7.1	<0.001†
I: 65 μsec, 600 Hz, 1 G‡	65.9 ± 19.9	64.8 ± 15.9	0.89 (NS)
II: 1 msec, 5 Hz, 0.02 G	24.9 ± 7.6	21.1 ± 4.4	0.15 (NS)
III: 1 msec, 5 Hz, 0.05 G	32.7 ± 10.1	22.6 ± 7.7	<0.001†
IV: 2 msec, 5 Hz, 0.05 G	37.9 ± 9.8	24.0 ± 5.5	<0.001†

PMF, pulsed magnetic field; ANOVA, analysis of variance; NS, not significant.
*I denotes signal I in phase 1; II, III, and IV denote signals II, III, and IV in phase 2.
†The difference in tensile strength versus control was statistically significant.
‡All wounds were treated for 21 days, except for wounds in this group, which were treated for 60 days.

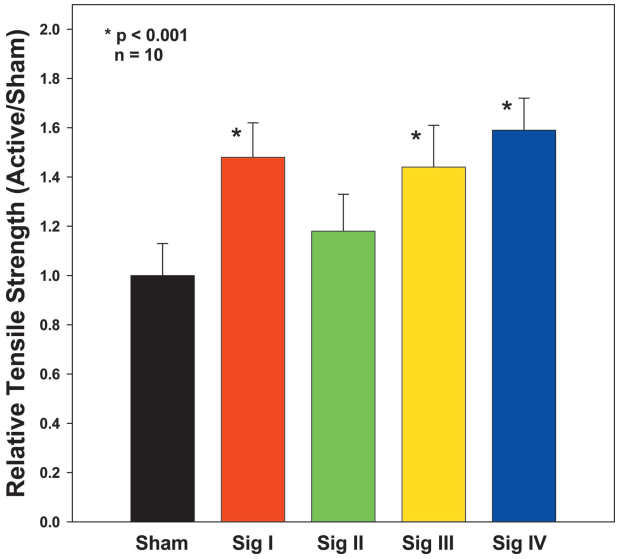

Fig. 1. Comparison of effects of different signal configurations on mean tensile strength of wounds at 21 days. Results are expressed as increase over sham. Signal I is used clinically for chronic wound repair. Signals II, III, and IV were configured assuming a Ca^{2+} binding transduction pathway. Signals I, III, and IV significantly accelerated wound healing, whereas signal II did not have sufficient amplitude to be effective.

ing growth factor-β.[33] It has also very recently been reported that pulsed magnetic fields, of the types used for bone repair, significantly increased endothelial cell tubulization and proliferation, as well as fibroblast growth factor β-2 in vitro.[26] In addition, there is recent evidence that their signals can modulate anti-CD3 binding at lymphocyte receptors, suggesting that pulsed magnetic fields can reduce the inflammatory response.[34] If these effects occur in this cutaneous wound model, accelerated healing would be expected, both from a reduction of time in the inflammatory phase and from subsequent acceleration of collagen production.

It is interesting to note that the production of growth factors has been reported to be calcium/calmodulin–dependent, and pulsed magnetic fields have been shown to accelerate Ca^{2+} binding to calmodulin.[35] In fact, the pulsed magnetic field signals utilized in phase 2 were configured assuming a calcium/calmodulin transduction pathway.[31,36] The model predicted that peak-induced magnetic field amplitude could be reduced from 1 G to the 0.01- to 0.05-G range, if the burst duration was extended from 65 μsec to the millisecond range. The results appear to confirm that increased burst duration allows the tissue amplitude to be substantially decreased. This, because the electric fields induced at tissue level to contain frequency spectra more closely matched to calcium/calmodulin binding kinetics.

The new pulsed magnetic field signals used in this study arose from the increasingly clear picture of the mechanism of pulsed magnetic field bioeffects.[31] Knowledge of the electrical properties of the transduction pathway, here chosen as Ca^{2+} binding to calmodulin, provided a quantitative basis for tuning the induced electric field to the transduction pathway. Since Ca^{2+} binding is a voltage-dependent (electrochemical) process with specific frequency characteristics, the signal must provide a detectable increase in voltage in the binding pathway to affect the rate of Ca^{2+} binding. This means the applied waveform must satisfy the frequency requirements of the binding process as well as apply a detectable increase in the voltage that drives it. Application of this model to the 65-μsec clinical signal shows it can be effective only at amplitudes in the vicinity of 1 G, primarily because the 65-μsec burst duration is too short to provide sufficient amplitude at the primary frequencies of Ca^{2+} binding kinetics. Although only a 1-G amplitude for signal I was examined in this study, it has been shown elsewhere that burst durations in the 65- to 100-μsec range are not effective in the 0.01- to 0.05-G range, even at repetition rates in the 500- to 1000-bursts/second range.[30,31] Clinical devices that use signal I are cumbersome and not portable, and are used primarily in the clinical setting. In addition, the power levels required to produce 1 G with this radiofrequency signal, which is in the short-wave radio band, are high enough to cause detrimental electrical interference in most medical monitoring equipment.

In contrast, the significantly longer burst duration of the new signals used in this study allowed the induced amplitude to be reduced by nearly 100-fold. This translates to a 10^4 decrease in power required to produce the signals, allowing battery-powered portability to be envisioned. Although the model predicted that a peak amplitude of 0.02 G could be effective at a burst duration of 1 msec, this did not prove quite sufficient, as the results for signal II demonstrate. Increasing the amplitude to 0.05 G rendered the signal significantly more effective, as shown by the results for signal III. A further increase in signal efficacy was obtained by increasing the burst duration to 2 msec (signal IV). These results provide support for the validity of the model and suggest further improvement in tissue effect may still be produced by varying the burst duration and perhaps the repetition rate in this model system.

It is interesting to compare these results with the report that the pulsed magnetic field signal widely utilized for recalcitrant fracture repair had no effect on the repair rate of a full-thickness wound model in the rat.[36] Although one of us (A.A.P.) developed this pulsed magnetic field waveform more than 30 years ago, it was not configured on the basis of the calcium/calmodulin transduction pathway; rather it was configured via a general electrochemical membrane model.[37] Indeed, frequency analysis of the bone repair signal in the calcium/calmodulin pathway reveals it to be severely mismatched to Ca^{2+} binding kinetics.

The mean wound tensile strength of pulsed magnetic field–treated and sham groups in phase 1 of this study was not statistically different at 60 days, although both groups exhibited an appropriate increase in tensile strength over time. This suggests that the healing wounds in the control group caught up with those in the treatment group, as the wounds in both groups reached completion of healing and finally approached the expected maximum mechanical strength at 60 days. This is characteristic of all reported pulsed magnetic field effects on tissue repair. For example, the pulsed magnetic field modulates bone repair by accelerating return to intact breaking strength and, therefore, function.[38] Sham-treated fractures reach the same biomechanical endpoint, as expected, but require more time to do so. Thus, pulsed magnetic field treatment has not been reported to make bone or skin stronger than normal tissue but rather to accelerate the progression to maximum strength. This is consistent with what is presently known about the mechanism of the pulsed magnetic field. Effects are at the cellular level, mainly through modulation of the expected cytokine and growth factor release in all stages of repair.[39] Pulsed magnetic fields accelerate repair but do not cause the wound to repair abnormally (i.e., go beyond the expected mechanical strength).

The results reported here serve to verify the healing properties of pulsed magnetic fields in a rat wound model. They establish a credible benchmark for testing pulsed magnetic field signal configuration (dosimetry) against a known efficacy. Our laboratory continues to investigate other signal configurations with this model.

CONCLUSIONS

We have successfully demonstrated that exposing wounds to radiofrequency pulsed magnetic fields appears to accelerate biomechanical healing (tensile strength). Comparison of the healing in the 21- and 60-day phase 1 groups suggests that tensile strength increased approximately 1.5 to 1.7 times faster in the pulsed magnetic field–treated groups versus the sham groups. The tensile strength at 60 days in the pulsed magnetic field–treated group was equal to that of controls at 60 days, as expected in this model. Treatment with pulsed magnetic fields does not make the wound stronger than expected at the end of the healing process, but it does make the wound stronger much earlier in the healing process.

This study suggests that it may be possible to increase the strength of a wound at an earlier phase in the postoperative period. In this day of early patient mobilization and early discharge from acute hospital care, this aspect of pulsed magnetic field therapy may prove to be extremely important.

Finally, our results suggest that this cutaneous wound model could be effective in investigations of variations in pulsed magnetic field signaling in wound repair. This model allows for convenient and rapid evaluation of the effect of pulsed magnetic field signal configuration as a dose parameter in wound repair. As our knowledge of cellular mechanisms increases, pulsed magnetic field signals will become more efficient and effective, and could be packaged in a more portable manner for clinical use.

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DISCLOSURES

Dr. Strauch is on the medical advisory board of Ivivi and owns stock in Ivivi. Ivivi partially supported the laboratory research. Dr. Pilla is a consultant to Ivivi. The other authors have no financial interests to disclose.

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