INTRODUCTION Induced alterations in bone’s innate bioelectric potentials play a role in de novo skeletal growth and development and in fracture repair [3, 5]. Although different models of pulsed electromagnetic field (PEMF) devices have been used to accelerate long bone non-union healing or to augment spine fusions [4, 9, 10], there is lack of consensus on the favorable influence of this treatment modality for bone fresh fractures [1, 9, 11]. Studies to document the beneficial role of PEMF on fresh fractures using animal osteotomy models have been published, but the reports are controversial [2, 6, 8]. We applied a rigorously controlled protocol and a highly sensitive in vivo micro-computed tomography (µCT) to unambiguously determine and quantify whether PEMF therapy stimulates significant changes in fresh fracture callus in the aged rat fibula. The protocol employed a commercially available and FDA approved PEMF waveform and a small (0.2 mm), mid-shaft, fibular osteotomy that mimicked a simple reduced fracture that would heal spontaneously within 5 to 6 weeks.

METHODS Animals and Operative Technique: 13 adult (9-11 months) male Sprague Dawley rats were anesthetized with Nembutal (60 mg/kg IP.). Bilateral mid-shaft fibular osteotomies were performed using a pre-cooled, high-speed rotary saw blade, according to standard procedures [7]. This created a gap of 0.2 mm in each fibula. Digital lateral plane radiographs were taken one day after surgery to verify the position and accuracy of the osteotomy. PEMF Treatment: Five days after surgery, each rat was placed in a custom sling-suit harness while under Isoflurane inhalation anesthesia. Each hind limb was fully extended and dangling outside the harness on each side of a centrally positioned shield made of an alloy with negligible magnetic reluctance. The right hind limb from the mid-thigh down was placed within the electromagnetic field generated by a custom PEMF coil, while the left hind limb was completely excluded from PEMF stimulation by the alloy barrier to serve as sham-treated internal control for each rat. The animals were exposed to an FDA-approved PEMF waveform, Physio-Stim® (Orthofix Inc., McKinney, TX), generated by a control unit powered by 9 V direct current power source. The magnetic field waveform consisted of bursts of triangular (saw-tooth) pulses having a pulse frequency of 3.8 kHz, a burst duration of 5.56 ms and a burst on-off period of 67 ms. The resulting burst on-off frequency was 15 Hz. The magnetic field amplitude was approximately 2 millitesla, equivalent to 20 gauss, was distributed uniformly over the fracture volume by virtue of a specially shaped coil. The coil enclosed a volume of approximately 7.5 cm (height) by 5 cm (width) by 2.5 cm (depth). The rats were exposed to this PEMF waveform for 3 hours a day, 7 days a week for 5 weeks. The waveform of each coil was checked routinely using a search coil [12] to ensure proper functioning of the coil, as well as the ability of the barrier to block PEMF penetration (Infinium Oscilloscope Model 5410A, Agilent Technologies, U.S.A.). Also, protocol compliance was ensured by regular inspection of both hind limbs. In vivo µCT Imaging: While under Nembutal anesthesia, and in supine position, both hind limbs were imaged simultaneously twice a week by in vivo µCT. The fibulae were segmented from the surrounding background signal. The time-sequence images were precision-registered to permit volumetric reconstruction of hard callus and woven bone newly formed within 5 mm proximal and distal to the osteotomy site. The PEMF-treated and sham-treated fibulae were analyzed with respect to the time of appearance, rate of formation and peak volume of hard callus achieved over the 5 weeks duration of treatment. After correcting for partial volume effects and the background signal, the callus volume reconstructed at each time point was indexed to the cortical bone volume on day 1 across the same 1 cm of bone around the osteotomy (Normalized Callus Volume).

RESULTS AND DISCUSSION Analysis of the normalized callus volumes with respect to days after osteotomy surgery from a total of 13 rats indicated a trend of 20-30% greater callus volume in PEMF-treated animals compared to sham-treated controls during all the postoperative period ($p = 0.059$). The between-limb difference with respect to the time of initial appearance of hard callus between PEMF- and sham-treated fibulae typically appearing around day 6 was not significant. Therewith, the rate of callus formation was linear over the next 10-15 days post-operatively (Fig. 1A). However, the 55% faster rate of hard callus formation for PEMF-treated in comparison to the sham-treated control fibulae was significant ($p = 0.023$) (Fig 1B). Basset et al., [2] analyzed the outcomes on fresh fracture of the radius in the rat using PEMF's of varying pulse characteristics. They concluded that differences in pulse characteristics were reflected in specific healing responses, although PEMF treatment was initiated 14 days after surgery. More recently, Leisner and others [8] evaluated a new PEMF waveform (PAP IMI) in fresh rat ulnar osteotomies and did not detect any positive response. In view of the fact that bone tissue changes due to PEMF stimulation may be rather subtle and uneven, such changes may be difficult to evaluate accurately based on traditional methods of 2D planar radiographs and histological tissue sections. The results from the present 3D study indicate a beneficial effect of Physio-Stim® PEMF on fresh fibular fracture healing during an early phase of callus formation and/or calcification. This effect appears to accelerate hard callus formation without significantly altering the extent of hard callus volume necessary to stabilize the fracture site.

![Image](image_url)

Figure 1 A: Rate of callus formation was determined by linear regression analysis of the normalized callus volume versus days after surgery for each fibula from each rat. Shown are the results for one rat in the study. B: Average rate of callus formation (mean ± SD) was calculated for sham- and PEMF-treated fibular groups for all 13 rats in the study.


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