Relevance to Musculoskeletal Conditions. Pulsed electromagnetic fields (PEMFs) are currently used to treat persistent nonunions and fractures with delayed healing. By understanding the mechanisms through which PEMFs regulate nonunion cells, it will be possible to optimize the osteogenic potential of this therapeutic modality.

Introduction. Although PEMFs are currently being used to treat persistent nonunions, relatively little is known about the underlying mechanisms responsible for their clinical success. Recent studies using animal and cell culture models indicate that PEMFs stimulate endochondral ossification by increasing production of transforming growth factor-beta-1 (TGF-β1) and cartilage mass (1-3). Osteochondroprogenitor cells are present in persistent experimental nonunions in canines (4), and these cells are responsive to TGF-β1 and BMP (5). These observations suggest that stimulation of TGF-β production may be an early step in PEMF-stimulated healing of nonunions in general. However, little is known about the cells present in human nonunions. The aim of the present study was to characterize cells present in human nonunion tissue and to examine the initial effects of PEMF on proliferation, differentiation, matrix synthesis, and local factor production.

Materials and Methods. Cells were isolated by enzymatic digestion from human hypertrophic (n=3) and atrophic (n=4) nonunion tissues (based on radiographic and histologic evaluation by two independent examiners). Confluent, second passage cultures were placed between Helmholtz coils and an electromagnetic field (5 ms bursts of 20 pulses repeating at 15 Hz) was applied for eight hours per day to one half of the cultures for one, two, or four days. Controls were cultured under identical conditions but without PEMF applied for eight hours per day to one half of the cultures for one, two, or four days. PEMF treatment/control (T/C) ratios were calculated by comparing values in the PEMF-stimulated or unstimulated cultures to values in the time 0 control cultures at each time point. Statistical differences were evaluated by use of the Wilcoxon signed rank test. P values < 0.05 were considered significant. Results were validated by running a second complete set of experiments in parallel.

Results. All nonunion cells had a fibroblastic appearance in culture. They exhibited moderate levels of alkaline phosphatase (> fibroblasts; < growth plate chondrocytes or osteoblasts), but they did not secrete osteocalcin into the media. All cells continued to proliferate in culture, produced a collagenous matrix, and secreted low levels of TGF-β1 and PGE2. PEMF caused a time-dependent increase in TGF-β1 release into the media of the cultures (Figure 1). Increases were noted in cultures of cells from hypertrophic nonunions by day two, while cells from atrophic nonunions did not show a significant increase until day four. PEMF had no effect on any of the other parameters examined.

Discussion. These results show that cells from human hypertrophic and atrophic nonunion tissues can be cultured in vitro. In addition, these observations support other reports showing that production of TGF-β1 is sensitive to electromagnetic field stimulation and that it is an early event in cell response. Hypertrophic nonunion cells may be more sensitive to PEMF in this regard because they are further along the osteochondral developmental pathway.

Figure 1: Effect of PEMF stimulation on TGF-β1 production by nonunion cell cultures. Panel A: TGF-β1 production by cultures from atrophic nonunions (n = 4 patients); Panel B: TGF-β1 production by cultures from hypertrophic nonunions (n = 3 patients). Treatment/control (T/C) ratios are shown for each panel and represent the mean ± SEM. *P<0.05, vs. T/C ratio of 1.0. Time 0 values were used for control values in determining T/C.

References

**Univ Texas Hlth Sci Ctr at San Antonio & Hebrew Univ, San Antonio, TX, & Jerusalem, Israel.
***ElectroBiology, Inc., Parsippany, NJ.
****Univ Texas Hlth Sci Ctr at San Antonio & Georg-August Univ, San Antonio, TX, & Goettingen, Germany.