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Basic Science

Pulsed electromagnetic field (PEMF) treatment reduces expression of genes associated with disc degeneration in human intervertebral disc cells

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Abstract

BACKGROUND CONTEXT: Pulsed electromagnetic field (PEMF) therapies have been applied to stimulate bone healing and to reduce the symptoms of arthritis, but the effects of PEMF on intervertebral disc (IVD) biology is unknown.

PURPOSE: The purpose of this study was to determine how PEMF affects gene expression of IVD cells in normal and inflammatory environments.

STUDY DESIGN/SETTING: This was an in vitro human cell culture and microarray gene expression study.

METHODS: Human annulus fibrosus (AF) and nucleus pulposus (NP) cells were separately encapsulated in alginate beads and exposed to interleukin 1α (IL-1α) (10 ng/mL) to stimulate the inflammatory environment associated with IVD degeneration and/or stimulated by PEMF for 4 hours daily for up to 7 days. RNA was isolated from each treatment group and analyzed via microarray to assess IL-1α- and PEMF-induced changes in gene expression.

RESULTS: Although PEMF treatment did not completely inhibit the effects of IL-1α, PEMF treatment lessened the IL-1α-induced upregulation of genes expressed in degenerated IVDs. Consistent with our previous results, after 4 days, PEMF tended to reduce IL-1α-associated gene expression of IL-6 (25%, p=.07) in NP cells and MMP13 (26%, p=.10) in AF cells. Additionally, PEMF treatment significantly diminished IL-1α-induced gene expression of IL-17A (33%, p=.01) and MMP2 (24%, p=.006) in NP cells and NFκB (11%, p=.04) in AF cells.

CONCLUSIONS: These results demonstrate that IVD cells are responsive to PEMF and motivate future studies to determine whether PEMF may be helpful for patients with IVD degeneration.

Keywords: Degeneration; Inflammation; IVD; Lower back pain; Matrix; PEMF

FDA device/drug status: Not approved for this indication (Orthofix Physio-Stim PEMF).

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The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

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Introduction

Chronic low back pain (LBP) due to degenerative disc disease is a significant cause of morbidity and is one of society's greatest expenditures of health-care dollars [1]. Although mechanisms of chronic LBP are not fully characterized, many symptoms are attributed to chronic inflammation [2–5] and impaired healing of disruptions within the matrix of intervertebral discs (IVDs) [6,7]. Cells from painful and degenerated discs have been shown to produce proinflammatory cytokines [3,8], including tumor necrosis factor α (TNFα) and interleukin 1α (IL-1α) [8,9]. These proinflammatory factors in turn stimulate catabolic degradation of collagen matrix via matrix metalloproteinases 2 (MMP2) [10] and 13 (MMP13) [11], both of which have been linked to painful disc degeneration [3]. These cytokines also further activate NF-κB [12] signaling to promote IL-6 [2,4,9,13] and IL-17 [14,15] expression within the disc, amplifying the proinflammatory feedback loop and exacerbating the catabolic behaviors within damaged disc tissue [2,16].

Conventional therapies for LBP, such as pain modulation and surgical intervention, address symptoms, but they often fail to target the fundamental causes of degeneration and pain, resulting in temporary or negligible therapeutic benefits [1]. Solutions or treatments that impede inflammation and matrix degeneration will likely improve clinical outcomes.

Pulsed electromagnetic field (PEMF) stimulation therapies have been clinically successful in treating a variety of medical conditions, including expedited healing of nonunion long bone fractures [17,18], increased fusion rate for cervical and lumbar fusions in high-risk patients [19–22], successful management of patients with discogenic lumbar radiculopathy [23], and arrested progression of osteoarthritis [24–29]. In addition, animal models have shown similar trends for osteoporosis [30]. Although researchers have demonstrated some clinical benefits of PEMF, the efficacy of PEMF is debated and the mechanism by which PEMF modifies cell behavior is not well understood. Additionally, thorough comparison of contradictory studies is obfuscated by the variety of PEMF waveform parameters. Despite this, a number of studies provide insight into the signaling pathways that mediate PEMF effects. An in vitro study of IVD cells demonstrated PEMF stimulated cell proliferation and suggested this occurred through the nitric oxide (NO) and prostaglandin E2 pathways [31]. Additional work has identified PEMF-induced upregulation of matrix synthesis through bone morphogenetic protein (BMP) signaling, particularly through BMP-2 and BMP-7 [17,29,32]. Additionally, unchanged levels of vascular endothelial growth factor and downregulation of IL-1β demonstrate that PEMF likely does not play a role in stimulation of hypoxic and inflammatory conditions [33].

Although previous studies show potential anabolic benefits from PEMF therapies, broad interpretations of the studies are limited. Additionally, it is unknown whether PEMF affects catabolic and proinflammatory cell behaviors consistent with painful disc degeneration. Thus, the primary goal of this study was to determine the effects of PEMF treatment on IVD cell gene expression in an in vitro model of IVD inflammation.

Methods

Intervertebral disc (IVD) cell isolation

We obtained both nucleus pulposus (NP) and annulus fibrosus (AF) tissue from 3 patients, NP tissue only from 2 other patients, and AF tissue only from another 2 patients (2 male, 5 female; age 50–82 years; average age 68 years; Pfirrmann grade ≤2) in accordance with an IRB-approved protocol (10–00786, University of California, San Francisco). Thus, a total of n=5 for each cell type and each condition. Samples were collected at the time of surgery in sterile collection tubes, transported on wet ice, and transferred into a sterile tissue culture hood. The IVD tissues of AF and NP were separated by blunt dissection and incubated separately in tissue culture flasks at 37°C, 5% CO_2 for 3–4 weeks. Cells were harvested by trypsinization, and passage 4 AF and NP cells were used for all experiments as previously described [34].

Alginate bead preparation

After trypsinizing cells, each cell type was individually resuspended in phosphate buffered saline 1.2% sodium alginate at a density of 2×10^6 cells/mL. Beads (approximately 4×10^4 cells/bead) were formed by dispensing the solution dropwise through a 22-gauge needle into a $CaCl_2$ cross-linking solution (102 mM). Twenty alginate beads were cultured in each well of a 6-well plate with 4 mL supplemented DMEM. All beads were acclimated to the medium for a 24-hour period before treatment.

Treatment conditions

IL-1α (10 ng/mL; Sigma-Aldrich Corporation, St. Louis, MO, USA) was used to stimulate the inflammatory environment associated with chronic LBP [35]. Cell-containing beads were exposed to an FDA-approved PEMF waveform (Physio-Stim, Orthofix Inc, Lewisville, TX) for 4 hours daily. The Physio-Stim waveform was powered by a 16 V direct current power source and consisted of a square wave with a 25% duty cycle, 3,850 Hz pulse frequency, 15 Hz burst frequency, and maximum 10 T/s rate of change. For quality control, a dB/dt sensor was used to monitor magnetic field during PEMF treatment. Culture media were replaced every 3 days, and samples were collected before PEMF dosing at 1, 4, and 7 days.

Cell viability

At each terminal incubation point (1, 4, and 7 days), the relative cell viability within a representative bead from each treatment group was assessed via Live/Dead assay (Life Technologies, Carlsbad, CA, USA) and quantified with ImageJ (NIH, Bethesda, MD, USA).

Microarray

At each terminal incubation point (1, 4, and 7 days), beads were placed in a 5× volume (960 μL) of dissolving solution (55 mM sodium citrate, 20 mM EDTA, 150 mM sodium chloride at pH 6.8), and gently agitated on a rocker for 15 minutes. The resulting solution was centrifuged for 10 minutes at 300 rcf, and the cell pellet was then washed with phosphate buffered saline (without Ca and Mg ions) twice. The cells were snap-frozen in liquid nitrogen and stored at −80°C until ready for processing. RNA was isolated from each sample using RNeasy Kits (Qiagen, Valencia, CA, USA) and stored at −80°C until analysis was performed.

Purified RNA was analyzed for quality using chip-based capillary electrophoresis (Bioanalyzer, Agilent, Inc., Santa Clara, CA, USA), and quantity and purity was determined with a NanoDrop spectrometer. The NuGEN Pico V2, based on Ribo-SPIA technology, was used for amplification, fragmentation, and biotin-labeling. The labeled cDNA was hybridized to Human GeneChip Gene 2.0 ST microarrays (Affymetrix, Inc., Santa Clara, CA, USA). The signal intensity fluorescent images produced during Affymetrix GeneChip hybridizations were read using the Affymetrix Model 3000 Scanner and converted into GeneChip probe results files (CEL) using Command and Expression Console software (Affymetrix).

Data analysis

Microarrays were normalized for array-specific effects using Affymetrix's Robust Multi-Array normalization. Normalized arrays values were reported on a log2 scale. For statistical analyses, we removed all array probe sets that had an average log2 intensity less than 3.0. This is a standard cutoff, below which expression is indistinguishable from background noise [36]. Linear models were fitted for each gene using the Bioconductor limma package in R [36,37]. Moderated t-statistics, fold-change, and the associated p-values were calculated for each gene. To account for the thousands of genes tested, we calculated false discovery rate-adjusted values using the Benjamini-Hochberg method [38]. False discovery rate values indicate the expected fraction of falsely declared differentially expressed genes among the total set of declared differentially expressed genes. p-Values less than .05 were reported as statistically significant.

Results

IL-1α induces an inflammatory response in IVD cells

Consistent with previous studies [35,39], treating AF cells with IL-1α for 4 days resulted in significant upregulation of various interleukins (IL-1α, IL-1β, IL-6) relative to controls (all with p<<.001; Table 1). In addition, at day 4, this study revealed that for AF cells treated with IL-1α, the following genes also experienced significant upregulation with respect to controls (p<.001 unless specified; Table 1): caspase 7 (CASP7), fibroblast growth factor 2 (FGF2), interferon gamma (IFNγ), MMP13, nerve growth factor (NGF), NF-κB, nitric oxide synthase (NOS2), TNFα, and TNFAIP6 (p=.001). Furthermore, AF cells treated with IL-1α for 4 days demonstrated significantly reduced expression of aggrecan (ACAN), collagen type 1 (COL1a1, COL1a2), FGF1, and transforming growth factor beta (TGFβ) (p=.04) compared with controls (p<.001 unless specified; Table 1).

Similarly, in NP cells, treatment with IL-1α for 4 days tended to upregulate expression of the following with respect to controls: FGF2 (p=.05), IL-1α (p=.08), NGF (p=.07), and TNFα (p=.09) and significantly upregulated CASP7 (p=.04), IL-1β (p=.02), IL-6 (p=.03), MMP2 (p=.3), MMP13 (p=.007), NF-κB (p=.04), and TNFAIP6 (p=.007). Although these

Table 1
Pertinent genes significantly affected by IL-1α after 4 days in AF and NP cells. Data have been normalized by their respective control group

		Fold change (IL-1α treatment/control)	
Gene	Gene name	AF	NP
ACAN	Aggrecan	0.4 (p<.001)	0.5 (p=.01)
CASP7	Caspase 7	2.3 (p<<0.001)	1.3 (p=.04)
COL1A1	Collagen type 1a1	0.5 (p<.001)	0.7 (p=.03)
COL1A2	Collagen type 1a2	0.3 (p<<0.001)	0.6 (p=.02)
FGF1	Fibroblast growth factor 1	0.4 (p<<0.001)	—
FGF2	Fibroblast growth factor 2	2.9 (p<<0.001)	1.5 (p=.05)
HAPLN	Hyaluronan proteoglycan link protein	—	0.6 (p=.05)
IFNγ	Interferon γ	2.2 (p<.001)	—
IL-1α	Interleukin 1α	212.0 (p<<0.001)	6.4 (p=.08)
IL-1β	Interleukin 1β	49.6 (p<<0.001)	2.1 (p=.02)
IL-6	Interleukin 6	13.7 (p<<0.001)	2.7 (p=.03)
MMP2	Matrix metalloproteinase 2	—	1.4 (p=.03)
MMP13	Matrix metalloproteinase 13	12.5 (p<<0.001)	3.7 (p=.007)
NF-κB	Nuclear factor κ-light-chain-enhancer of activated B cells	3.5 (p<<0.001)	1.7 (p=.04)
NOS2	Nitric oxide synthase 2	20.2 (p<<0.001)	—
NGF	Nerve growth factor	8.6 (p<<0.001)	1.9 (p=.07)
TGFβ	Transforming growth factor β	0.7 (p=.04)	—
TNFα	Tumor necrosis factor α	6.6 (p<<0.001)	1.6 (p=.09)
TNFAIP6	Tumor necrosis factor α interacting protein 6	3.1 (p=.001)	1.8 (p=.007)

changes were not as substantial as those seen in AF cells, they were still statistically significant (Table 1). Additionally, compared with controls, ACAN (p=.01), COL1a1 (p=.03), and COL1a2 (p=.02) were significantly downregulated, and hyaluronan and proteoglycan link protein 1 (HAPLN) (p=.05) tended to be downregulated in IL-1α-treated NP cells (Table 1).

PEMF does not affect cell viability

In both cell types, PEMF did not significantly alter mRNA expression of genes associated with cell cycle or apoptosis in either normal culture or IL-1α-treated groups at any time point (Appendix 1). Likewise, in both media conditions and across all time points, PEMF did not have a detectable effect on AF or NP cell viability as determined by Live/Dead staining (Figure).

Pulsed electromagnetic field reduces, but does not completely prevent, expression of IL-1α-induced expression of inflammatory cytokines in IVD cells

Pulsed electromagnetic field downregulated IL-1α (p=.01), IL-1β (p=.03), and NF-κB (p=.04) expression in AF cells in IL-1α-supplemented media for 4 days compared with AF cells treated with IL-1α alone (Table 2). In NP cells, PEMF significantly diminished IL-1α-induced expression of IL-17A after 4 days (p=.01), and, as previously shown [39], PEMF tended to reduce IL-1α-associated expression of IL-6 in NP cells (p=.07; Table 2). Although PEMF treatment reduced the expression of proinflammatory markers in IVD cells treated with IL-1α relative to those treated only with IL-1α, it did not entirely counteract the effects of IL-1α; the IVD cells treated with IL-1α and PEMF tended to express higher levels of proinflammatory genes than IVD cells cultured in control conditions. Additionally, PEMF resulted in no significant upregulation of proinflammatory genes in IVD cells cultured in IL-1α-supplemented media or control media (Appendix 2), nor did it affect the expression of natural inflammation inhibitors TNFAIP6, IL-4, IL-10, IL-11, or IκB in any conditions (Appendix 3). By day 7, groups treated with PEMF and IL-1α did not significantly differ from groups treated with IL-1α alone.

PEMF reduces, but does not completely inhibit, expression of matrix catabolizers in proinflammatory conditions

After 4 days, PEMF tended to reduce IL-1α-associated MMP13 expression in AF cells (p=.10) and MMP2 expression in NP cells (p=.006; Table 2). Intervertebral disc cells treated with IL-1α and PEMF tended to express higher levels

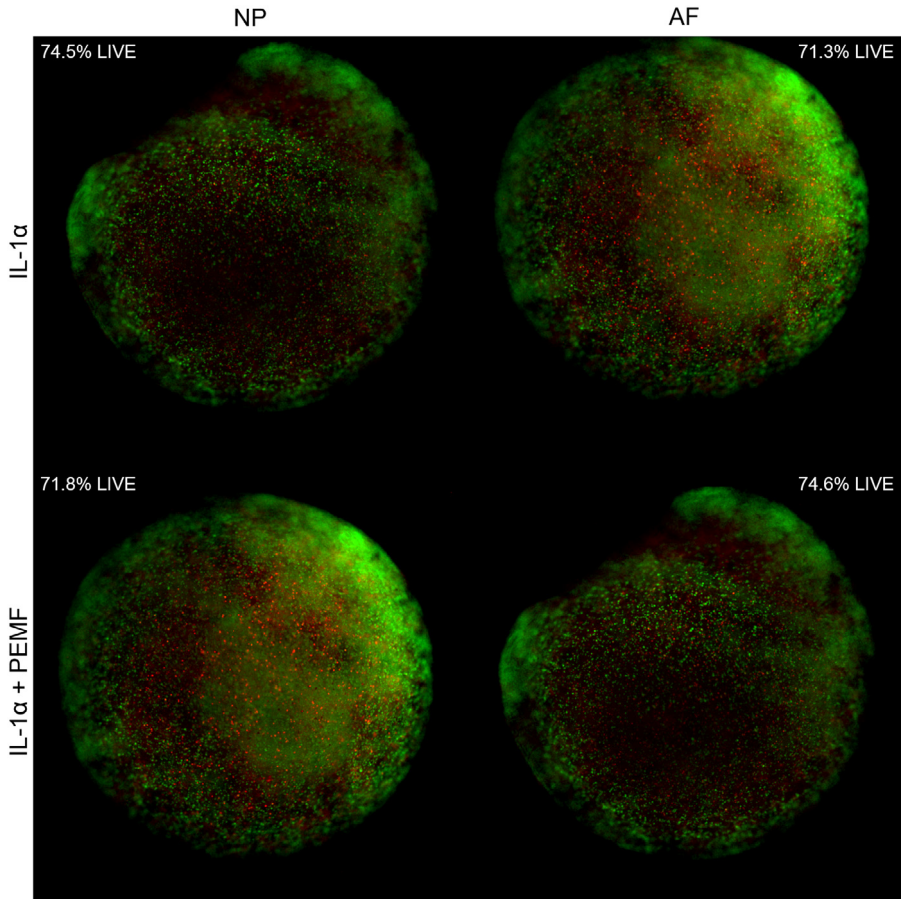

Figure. Representative images from Live/Dead staining of IVD cells in alginate beads after 7 days of treatment (green: live, red: dead).

Table 2
Pertinent genes significantly affected by PEMF in proinflammatory conditions after 4 days in AF and NP cells. Data have been normalized by their respective control group

Gene	Gene name	Fold change (PEMF&IL-1α treatment/IL-1α treatment)	
		AF	NP
FGF1	Fibroblast growth factor 1	1.3 (p=.04)	—
HAPLN	Hyaluronan proteoglycan link protein	—	1.3 (p=.07)
IL-1α	Interleukin 1α	0.45 (p=.01)	—
IL-1β	Interleukin 1β	0.43 (p=.03)	—
IL-6	Interleukin 6	—	0.89 (p=.07)
IL-17	Interleukin 17	—	0.72 (p=.01)
MMP2	Matrix metalloproteinase 2	—	0.75 (p=.006)
MMP13	Matrix metalloproteinase 13	0.64 (p=.10)	—
NF-κB	Nuclear Factor κ-light-chain-enhancer of activated B cells	0.90 (p=.04)	—

of catabolic genes than IVD cells cultured in control conditions. Pulsed electromagnetic field caused no significant effect on expression of MMPs in control conditions (Appendix 2). By day 7, the groups treated with PEMF and IL-1α did not significantly differ from groups treated with IL-1α alone.

PEMF induces expression of factors that are conducive to regeneration of matrix

In addition to the inhibition of genes responsible for matrix degradation under proinflammatory conditions at day 4, PEMF plus IL-1α treatment tended to reduce expression of genes required for healthy matrix formation relative to groups treated with IL-1α alone, including HAPLN (p=.07) in NP cells and fibroblast growth factor 1 (FGF1; p=.04) in AF cells (Table 2). Pulsed electromagnetic field treatment of IVD cells under proinflammatory conditions returned the expression levels of the aforementioned genes to those seen in control conditions. However, PEMF had no significant effect on matrix proteins, such as ACAN and COL1, in control or proinflammatory conditions (Appendix 4). By day 7, the groups treated with PEMF and IL-1α did not significantly differ from groups treated with IL-1α alone.

Discussion

The purpose of this study was to determine the effects of Physio-Stim PEMF on IVD cell gene expression in a simulated proinflammatory in vitro environment. In both NP and AF cell types, we observed that gene expression associated with the proinflammatory and extracellular matrix catabolism cascades were the most clinically relevant signaling pathways constructively affected by PEMF.

Consistent with previous studies, IVD cells treated with IL-1α expressed significantly more proinflammatory genes than controls at day 4 [35,39]. Proinflammatory cytokine expression and the associated signaling cascades are indicative of disc degeneration and discogenic pain [3]. NF-κB is a transcription factor heavily linked to the initiation and progression of inflammatory processes in IVDs [12], including the IL-1, IL-6, and TNFα signaling cascades. IL-1α, IL-1β, and IL-6 further upregulate TNFα. Tumor necrosis factor alpha is a proinflammatory adipokine that negatively affects chondrocyte proliferation and matrix formation, and it has been linked to IVD matrix degeneration [8,9]. In degenerated discs, TNFα upregulates TNFAIP6 [40,41] and IL-17 [14,16]. TNFAIP6 is a part of a negative feedback loop that limits inflammation and reduces matrix degradation through its interaction with matrix proteins and its inhibition of matrix catabolic factors [40,42]. IL-17, in turn, is expressed following cartilage injury, and it promotes the production and release of proinflammatory mediators prostaglandin E2 and NO [15].

At 4 days, PEMF significantly diminished IL-1α-associated gene expression of NF-κB, IL-1α, IL-1β, and tended to reduce IL-6 gene expression. Pulsed electromagnetic field treatment also reduced IL-17 gene expression, but not TNFAIP6 gene expression in proinflammatory conditions. Together, these results indicate that PEMF has the ability to reduce the effect of IL-1α treatment on proinflammatory gene expression at early stages of inflammation. However, because no significant effects were observed at later time points, PEMF may only be useful to inhibit early proinflammatory signaling [43]. Pulsed electromagnetic field has been shown to modulate the rate of calmodulin (CaM) activation through the acceleration of calcium ion binding kinetics, thus increasing the CaM-dependent NO release from stressed cells [44]. Injury-induced calcium influx and NO release is transient; thus, the observed short-term effects of PEMF in IL-1α-treated IVD cell cultures may be tied to the transient nature of the NO cascade [31,45,46].

A hallmark of disc degeneration is loss of matrix and decreased capacity of IVD cells to repair matrix components. There is extensive literature on the link between IVD degeneration and extracellular matrix degradation through matrix metalloproteinases [47], including increased levels of both MMP2 and MMP13 [10]. MMP2 and MMP13 play a role in the catabolic breakdown of collagen and are active in many IL-1α- and TNFα-linked inflammatory processes. Pulsed electromagnetic field reduced IL-1α-induced MMP2 and MMP13 expression in IVD cells at 4 days. These results highlight a role for PEMF in the abatement of IVD matrix breakdown in early stages of inflammation. Additionally, PEMF treatment resulted in a slight upregulation of HAPLN and FGF1

in proinflammatory conditions. Hyaluronan and proteoglycan link protein 1 is a link protein key to the formation and structural integrity of cartilaginous matrices [48], and FGF1 is a growth factor linked to chondrocyte proliferation and proteoglycan synthesis [49,50]. The loss of these proteins have been linked to disc degeneration [48,50].

Because PEMF affected genes key to both matrix formation and cell growth, PEMF treatment may be slightly regenerative. However, despite other PEMF waveforms having been shown to stimulate IVD cell matrix regeneration [32], in our study, PEMF did not stimulate ACAN or COL1 production in any media condition, implying that PEMF treatment does not completely regenerate damaged matrix. Given the extent of choices in PEMF parameters, this variation in study outcome emphasizes the need to determine if there is also an optimal signal and dosing that would accelerate both disc regeneration and proinflammatory inhibition. Thus, the current PEMF signal and dosing may mainly have some potential as a therapy to decelerate disc degeneration in a subset of patients with acute and mild conditions. Only clinical studies will help determine the best indication for this therapy and whether there are cofactors that could strengthen the PEMF's ability to regenerate the disc.

Although only relatively healthy IVD samples (Pfirrmann grade≤2) were used for this study, there was a high degree of variability in gene expression patterns between donated tissues. This between-subject variability, combined with a low sample number (n=5), limited our analysis and interpretation of the effects of PEMF. Yet, despite this variability, we observed statistically-significant PEMF effects on several important inflammatory factors, indicating the disease-modifying effect of this particular PEMF signal is mostly anti-inflammatory, anti-catabolic rather than matrix synthetic. Additional mechanistic studies are currently underway to better define the time course of PEMF effects and specific cell phenotype markers that relate to PEMF reactivity. These studies may clarify the basis for the observed donor-to-donor variability and may be used to better define patients who would benefit from this therapy. An additional limitation is that this in vitro study relied on IVD cells that were removed from their native environment, thus confounding potential clinically relevant factors contributed by innate structural elements, mechanical loading, and surrounding tissues. Although this study did not account for any potential dampening of magnetic field by soft tissue, other studies have shown that PEMF waveforms of similar intensity do penetrate the body and are effective in the context of the spine [19]. Additionally, these results are only indicative of gene expression changes at the transcriptional level, and any interpretations of the changes in signaling cascades are merely speculative. Thus, even though we detected gene transcription changes in IVD cells, translating these effects to a potential clinical effect on IVDs would be extrapolative at this point.

Physio-Stim PEMF treatment had significant effects on the expression of genes associated with early stages of inflammation and some effects on genes associated with matrix degradation, but PEMF had minimal effects on matrix repair and cell growth. The short-term effects of PEMF on proinflammatory cytokine and MMP expression highlight a potential role for PEMF in the treatment of acute inflammation in IVDs and motivate further studies to examine post-transcription effects in addition to optimization of the PEMF signal and dosing to improve the extent and duration of these potential benefits for patients with IVD degeneration and LBP.

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Appendix: Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.spinee.2016.01.003.

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