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Focused-pulsed electromagnetic field treatment reverses lipopolysaccharide-induced alterations in gene expression profile in human gastrointestinal epithelial cells

Asit Panja, Rolf Binder¹, Silvia Binder²

Abstract

BACKGROUND: Although the therapeutic effects of pulsed electromagnetic field (PEMF) treatment are well documented, underlying mechanisms of PEMF in treating pathological conditions are incompletely understood.

METHODS: We utilized a human gastrointestinal epithelial cell system to investigate the influence of a low-frequency electromagnetic field generated from a focused PEMF (f-PEMF) device on the expression of human genes. We simulated an inflammatory condition by stimulating the cells with lipopolysaccharide (LPS). A set of LPS-activated cells were then subjected to 100-Hz f-PEMF for 30 seconds to observe the therapeutic effect of f-PEMF. We determined the therapeutic effect by analyzing the reversal of LPS-induced alterations in gene expression using RNA-seq analysis. The results were compared to the changes between untreated controls versus LPS treated cells, defining the homeostatic alteration of changes in gene expression profile caused by LPS stimulation alone. We further compared LPS treated versus LPS + f-PEMF treated cells to examine the effect of f-PEMF in the reversal of the LPS-induced alterations in gene expression patterns.

RESULTS: A total of 38,162 genes (of 60,448 tested) were constitutively expressed in the untreated control cells. Stimulation with LPS altered the expression profile through de novo-induction of >1950 genes that were originally unexpressed and silencing 2486 constitutively expressed genes. LPS treatment also altered expression levels in a large panel of genes. Exposing LPS-treated cells to 100 Hz of f-PEMF for 30 seconds (s) showed reversals of LPS treatment-induced altered gene expression. In this paper, we emphasize the f-PEMF regulation of genes associated with inflammatory processes.

CONCLUSION: Our data indicates for possibility of developing new nonchemical alternative therapeutic approaches for treatment of inflammation and pain.

Keywords:

Gastrointestinal epithelial cell, gene expression, inflammation, lipopolysaccharide, pulsed electro-magnetic field

Introduction

Advances in medical sciences during the past century have meaningfully shifted the nature of disease landscape and treatment modalities for many

diseases. Acute illnesses caused by specific infectious microorganisms are being replaced increasingly by chronic disorders with multiple etio-pathophysiological factors. This emerging trend of chronic inflammatory health conditions arises

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AlfaGene Bioscience
Inc. Fords, New Jersey,
USA, ¹ONDAMED GmbH,
Schwanau, Germany,
²The Binder Institute for
Personalized Medicine,
Schwanau, Germany

Address for correspondence:

Dr. Asit Panja,
Alfagene Bioscience Inc.,
20 Corrielle Street, Fords,
New Jersey 08863, USA.
E-mail: asit.panja@alfagenebioscience.com

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from complex interactions among cellular, molecular, genetic components of various organs and tissues in the body, which are further influenced by environmental factors.^[1-5] Inflammation is also caused by direct injuries such as trauma or infection to the tissues and associated organs.^[6-8] Inflammation remains the major cause of many chronic illnesses.^[9-11] In many cases, inflammation-mediated illnesses are diagnosed and treated based on symptoms and signs within a single system or organ rather than the variables affecting the disease pathogenesis. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) are often used to block the effects of inflammatory mediator prostaglandin produced by vascular components in response to inflammation in the localized tissues.^[12-15] While such symptomatic treatments alleviate symptoms, they do not target homeostatic dysbalances in the inner core of the cellular components of the affected tissues, organs, and/or the systems. Many of such anti-inflammatory drugs used for treating these conditions also cause severe side effects.^[14,16,17] In addition, the cost of these medications is expensive, especially when the treatment is required for a long period.^[18-21] Therefore, health care providers have an obligation to overcome these challenges by exploring alternative therapeutic approaches which would be safe, effective, rapid, and without any invasive risks.

Electromagnetic (EM) therapy has been known to stimulate the body's own healing mechanisms by regulating intrinsic mechanisms for repair and regeneration of the pathological state of selected body areas or weakened tissues^[16,22-27] possibly, through restoration and/or realignment of the subcellular components and/or the quantum energy field inside the cells (Cyto-Quantum-Energy). Thus, EM and other alternative therapy are increasingly in demand by physicians and patients alike. However, despite its historical use and modern popularity, a considerable level of wariness exists in the integration of such concepts into modern medicine. This is largely in part due to the lack of understanding of how the low-frequency EM impulses interact with the cellular, molecular, genetic components and regulate the function of specific organs and/or tissues in the human body.

Therefore, we have recently initiated a multi-disciplinary collaborative program to study bio-interactive mechanisms of focused EM fields with a human gastrointestinal (GI) stem cell-like primary epithelial cell system and a pulsed electromagnetic field (PEMF) device using focused EM fields. The results from a pilot study demonstrating the effect of pulsed EM stimulation on gene expression profile and its relevance to potential mechanisms of therapeutic effects on chronic inflammatory conditions are discussed in this paper.

According to the results, LPS induced changes in the inflammation associated gene expression profile in human cells is reversible by optimum focused PEMF treatment, perhaps, through restoration and/or reorganization of the quantum energy field inside the cells (cyto-quantum-energy-homeostasis) used in this study. The reported concept of cyto-quantum-energy-homeostasis for gene expression regulation by focused PEMF opens new possibilities for the development and utility of devices that may re-establish the physiological environment in cells, organs, and tissues of inflamed or chronically damaged areas in the body.

Materials and Methods

The aim of this study is to determine the mechanisms of action of focused PEMF (f-PEMF) treatment in cellular, molecular, and genetic levels in the human body. Emphasis has been on the inflammatory panel of genes. An *in vitro* model of an inflammatory scenario was established by stimulating a GI stem cell-like human intestinal primary epithelial cell population^[28,29] with lipopolysaccharides (LPS) (Sigma, St Louis, MO). LPS is a cell wall protein from Gram-negative bacteria and is known for its inflammatory effects in humans.^[30-33] In many acute and/or chronic inflammatory conditions of the GI tract, epithelial barrier function is thought to be disrupted by LPS. This further leads to the activation of immunoresponsive cells in the gut, causing overproduction of inflammatory cytokines (cytokine storm).^[34] To examine whether and how focused PEMF could influence the LPS altered molecular biology of the cells, in a separate condition, the same LPS stimulated cells were further exposed to a focused EM field of 100 Hz for 30s by using the ONDAMED device [Figure 1]. Cells were then cultured for an additional 14 h in a humidified CO₂ incubator. Gene expression profile in all three conditions (untreated, LPS treated, and LPS + ONDAMED' focused PEMF) were analyzed using RNAseq analysis through a third-party laboratory.

Cell culture

A frozen vial of 500,000 GI stem cell-like primary epithelial cells (from AlfaGene Bioscience Inc, Fords,



Figure 1: Pulsed electro-magnetic frequency exposure to lipopolysaccharide treated gastrointestinal epithelial cells in culture

NJ, USA) was defrosted, washed, and cultured in 3 ml of HIPEC medium^[28,29] in a vented-capped 25 cm² sterile tissue culture flask (Corning, NY, USA) at 37°C temperature in a humidified (90%) CO₂ (5%) incubator. Upon 80% confluency, cells were trypsinized and dissociated from the culture flask, washed three times in F12 medium. Cells were then re-seeded in 6-well tissue culture plates (7.5 × 10⁵ cells/2 ml of medium/well) for the experiment, as described above in Figure 1. Three separate plates were used for each of the three conditions (1. medium alone, 2. medium + LPS 1 mg/ml, and 3. medium + LPS + PEMF) and placed in three separate racks in the incubator for 14 h after the addition of LPS (conditions 2 and 3) and PEMF (condition 3 only) treatment.

Ondamed's focused-pulsed electromagnetic field exposure

PEMF exposure to the cultured cells was carried out by using a focused PEMF treatment device developed by the Ondamed Company (Schwanau, Germany). The device, invented in Germany in 1993 by electronics engineer Rolf Binder, employs the mechanism of action of pulsed EM fields emitted through various applicators. Four differently sized applicators deliver targeted stimulation to a localized area while a fifth available applicator delivers a wide field stimulation providing a systemic therapeutic effect such as the lymph system, bone, and joints. Frequencies range between 0.1 and 31,835 Hz with an available intensity setting between 0.5 and 55 mT (milli Tesla). The field density varies for each applicator.

An EM field was created by placing an applicator with a 2.4" (6 cm) distance for stimulation connected to the PEMF generating device on a flat surface of a wooden countertop at room temperature. A bridge of approximately 2.4" (6 cm) height was created by placing a plastic box on both horizontal sides of the EM matrix applicator. Then, a holding bridge was created by placing another container on the top of the surface, as shown in Figure 1.

The 6-well cell culture plate containing cultured human intestinal primary epithelial cells (750K cells/2 ml of medium/well) were treated with LPS (1 µg/ml) 10 min before the EM exposure was placed on the top of the bridge surface. This set up allowed vertical transmission of EM waves (100 Hz) to the center top of the designed bridge. The device contains control switches for an EM frequency intensity as well as exposure time. LPS-stimulated cells were subjected to a single exposure of 100 Hz PEMF for 30s followed by their placement in the incubator as described above in the cell culture section.

RNA extraction

After 14 h of (posttreatment) incubation period, medium from the cultured cells was collected, centrifuged to obtain cell-free supernatants and stored at -20°C for future analysis for secreted proteins. At the same time, the adhered cells in the wells of the culture plates were gently washed with PBS, followed by lysis in RNA lysis solution. Total RNA was extracted from these cell lysate samples from each of the three experimental conditions by using the RNeasy kit (Qiagen, Germantown, MD) following the manufacturer's instructions. The final RNA pellet was dissolved in RNase-free water. The yield of total RNA from each of three conditions (with the equal number of seeded cells) was 13 µg (medium only), 2 µg (medium + LPS), and 9 µg (medium + LPS + f-PEMF), respectively. These results from total RNA yield itself indicated that the reduction in the amount of total RNA from the LPS treated cells might have been due to inhibition of cell growth or induction of cell death by LPS treatment to these primary epithelial cells, which was overcome by f-PEMF treatment.

Extracted total RNA samples were labeled in a coded fashion and sent for further quality assessment and subsequent RNAseq analysis to an FDA compliant Next Generation Sequence (third party contract research organization) laboratory.

RNAseq and gene expression analysis

RNAseq analysis was performed using CLC Genomics Workbench v. 10.0.1. (Qiagen, Redwood City, CA). Illumina reads were trimmed to remove low-quality ends and adapter sequences. Sequences of at least 50 bp in length were then mapped to the GRCh38 reference genome using the RNA-Seq Analysis tool in CLC. Total gene and transcript hit counts were measured, and RPKM (read per kilobase million) values were calculated. Gene and transcript values were analyzed separately. Kal's Z-test was used to compare the expression values in different conditions. Genes with $P \leq 0.05$ and absolute proportions fold change ≥ 2 were called as differentially expressed genes for each comparison. Transcripts with $P \leq 0.05$ and absolute proportions fold change ≥ 2 were called differentially expressed transcripts for each comparison. Gene results were annotated with the Gene Ontology Biological Process information. Then, Hyper-G tests for each comparison were conducted using the genes with a fold change >2 and $P < 0.05$.

Results

To gain insights on how f-PEMF may act in cellular constituents of tissue in various pathological settings, we took a simple approach by asking the question of what changes occur at the genomic level of a cell during the initiation and perpetuation of an inflammatory process

and then whether PEMF treatment can have any role in reversing the changes if there are any [Tables 1 and 2]. Hence, the first step to address this question was to assess the changes in the total number of expressed versus unexpressed genes caused by LPS alone or LPS + PEMF compared to the untreated (medium only) cells. As can be seen in Table 1, out of total 60,448 genes tested, only 38,162 genes were constitutively expressed in the untreated cells (medium only). This number of expressed genes remained in balance (almost unchanged) after LPS stimulation (38,164 genes), which was however, reduced by 2,932 genes on exposure to focused PEMF treatment (35,232 genes). The observed balance in the total number expressed or unexpressed genes in control (medium only) versus LPS treated cells raised two possibilities: (i) LPS may not have any effect in turning on or off any genes in these cells but may have regulatory effects only on the expression levels or (ii) The number of genes induced (turned on expression) by signals from LPS stimulation was balanced by silencing the equal number of genes (turning off the expression) along with its effects on the expression levels.

Therefore, to rule in or rule out any of these possibilities, we counted the total number *de novo* expressed genes (that were not expressed in the untreated) and the number silenced (that were originally expressed in untreated cells) in both LPS treated and LPS + PEMF treated cells compared to the control condition (medium only). *De novo* expression of at least 1,950 genes in LPS treated and 1,371 genes in LPS + focused PEMF treated cells were observed. Similarly, there were 2,486 and 4,303 genes were turned off by LPS alone and LPS + PEMF treatment, respectively [Table 2]. Similar analytical observation was made in the transcriptome profiles as well. However, as mentioned above in this paper, our focus is only on the gene expression profile with special emphasis on the inflammation-related genes. Transcriptome data, as well as the analysis of other functional groups of genes, will be reported in subsequent papers from our group.

Our next goal was to further explore whether and how PEMF regulates actual levels of expression of various functional groups of genes that are altered by disease initiating processes. For this purpose, we created at least 12 functional groups of genes filtered from the genome database by using CLC software. Then we further filtered and organized the list of genes in two groups. One with the list of genes of which the expression levels were increased by LPS treatment. Similarly, the second group included the genes that were decreased by LPS stimulation compared to the baseline control. We display and compared side-by-side the RPKM values (measure of expression level) of all the genes from each of the three experimental conditions (medium only, LPS stimulated, LPS stimulated + focused PEMF treated) for all the 12 functional groups (not shown in this paper). Results from the functional module of inflammation-related genes are shown in Tables 3 and 4. As can be seen in both of these tables, PEMF treatment had a profound effect in reversing the altered expression levels irrespective of the signaling directions (up-regulatory or down-regulatory) by LPS. Similar pattern of genes expression regulation by PEMF was seen in the other functional groups (e.g. cell growth, differentiation, apoptosis, sensory, immunoregulatory, cytokines, chemokines, etc.). These observations suggest that an optimized level of EM energy may have a significant impact on biological systems.

Most interestingly, we have noted that 23 genes were regulated with statistical significance ($P = 0.05$). Our findings not only substantiate the therapeutic potential of PEMF through genomic homeostasis regulation but also provide valuable information that PEMF treatment could potentially offer an alternative solution to safety concerns of currently marketed anti-inflammatory drugs such as NSAIDs, which acts through inhibition of PSTG gene but bears a wide range of side effects. However, before considering PEMF as an alternative to NSAIDs, it is important to determine if there is any potential toxicity caused by PEMF exposure in our experiments. Therefore,

Table 1: Changes in total number of expressed versus unexpressed genes in LPS treated versus LPS + PEMF treated cells

Status	Control (medium only)	+ LPS (1 µg/ml)	+ LPS (1 µg/ml) + PEMF
Expressed genes (n)	38,162	38,164	35,232
Unexpressed genes (n)	22,282	22,287	25,292

Genes with RPKM value greater than zero is considered as expressed and those with RPKM value of zero or less is considered as unexpressed genes. RPKM=Read Per Kilobase Million, LPS=Lipopolysaccharide, PEMF=Pulsed electro-magnetic field.

Table 2: Total number of genes turned-on (induced) or turned-off (silenced) by LPS treatment or LPS + PEMF treatment compared to baseline (unstimulated/untreated) control condition

Status	+ LPS (1 µg/ml)	+ LPS (1 µg/ml) + PEMF
Number of <i>de novo</i> expressed (turned-on) genes	2,486	4,303
Number of silenced (turned-off) genes	1,950	1,371

Genes that were not detected (RPKM value=0) in untreated cells (cultured in medium only - baseline control) but appeared to have RPKM value greater than zero in treated conditions are considered to be Turned-on (induced) and those which had a RPKM value of greater than zero in the baseline control condition but were not detected (RPKM value zero) were considered as silenced genes. RPKM=Read Per Kilobase Million, LPS=Lipopolysaccharide, PEMF=Pulsed electro-magnetic field.

Table 3: Pulsed electro-magnetic frequency treatment reverses upregulated expression of inflammation associated genes in LPS -stimulated human gastrointestinal stem cell-Derived epithelial cells

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated Cells	RPKM value after EM treatment on LPS treated Cells
PARP4	Poly (ADP-ribose) polymerase family member 4	NAD+ADP-ribosyltransferase enzyme binding	19.953	22.327	19.481
NFKBIZ	NFKB inhibitor zeta		7.699	9.246	8.314
IVNS1ABP	Influenza virus NS1A binding protein		6.172	6.581	5.347
PTGS2	Prostaglandin-endoperoxide synthase 2	Prostaglandin-endoperoxide synthase activity; arachidonate 15-lipoxygenase activity; lipid binding	5.912	6.143	3.579
THBS1	Thrombospondin 1	Heparin binding; low-density lipoprotein particle binding; integrin binding; phosphatidylserine binding; extracellular matrix binding; fibronectin binding; proteoglycan binding; fibroblast growth factor binding; collagen V binding; transforming growth factor beta binding; laminin binding calcium ion binding	5.712	6.841	4.105
PARG	Poly (ADP-ribose) glycohydrolase	Poly	5.046	5.318	4.872
DCP2	Decapping mRNA 2	Hydrolase activity; manganese ion binding; rna binding; exoribonuclease activity, producing 5'-phosphomonoesters; m7G; protein binding	4.969	5.472	5.232
ACVR1	Activin A receptor Type 1	Activin receptor activity, type I; ATP binding; transforming growth factor beta receptor activity, type I; receptor signaling protein serine/threonine kinase activity; SMAD binding; protein Homodimerization activity	3.992	4.880	4.663
UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats	Protein binding	3.852	4.062	3.728
VPS16	VPS16, CORVET/HOPS core subunit	Actin filament binding	3.530	3.681	3.553
LTA4H	Leukotriene A4 hydrolase	Leukotriene-A4 hydrolase activity; zinc ion binding; epoxide hydrolase activity; aminopeptidase activity; metallopeptidase activity	2.861	2.881	2.861
ITGA2	Integrin subunit alpha 2	Protein heterodimerization activity; viral receptor activity; metal ion binding; protein binding; laminin binding; collagen binding	2.601	3.326	2.246
PNMA1	Paraneoplastic Ma antigen 1	Protein binding	2.429	2.532	2.327
MAP2K3	Mitogen-activated protein kinase kinase 3	Protein tyrosine kinase activity; transferring phosphorus-containing groups; MAP kinase kinase activity; receptor signaling protein serine/threonine kinase activity; protein binding; ATP binding; ATP binding	2.367	2.641	2.127
ROCK1	Rho associated coiled-coil containing protein kinase 1	Serine/threonine kinase activity; ATP binding; metal ion binding; Rho GTPase binding	2.025	2.252	2.130
ITGB6	Integrin subunit beta 6	Integrin binding; viral receptor activity	2.014	2.061	2.050
F3	Coagulation factor III, tissue factor	Phospholipid binding; protein binding; protease binding	1.989	2.476	1.724
CD44	CD44 molecule	Transmembrane signaling receptor activity; hyaluronoglucosaminidase activity; collagen binding	1.944	2.344	2.299
BMP2	Bone morphogenetic protein 2	Receptor binding; cytokine activity; SMAD binding; phosphatase activator activity; heterodimerization activity; retinol dehydrogenase activity; BMP receptor binding	1.569	1.835	1.546
TTBK2	Tau tubulin kinase 2	ATP binding; protein serine/threonine kinase activity	1.521	2.007	1.787

Contd...

Table 3: Contd...

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated Cells	RPKM value after EM treatment on LPS treated Cells
KDM6B	Lysine demethylase 6B	Metal ion binding; dioxygenase activity; sequence-specific DNA binding; histone demethylase activity	1.418	1.682	1.544
IL20RB	Interleukin 20 receptor subunit beta	Interleukin-20 binding	1.398	1.421	0.775
NFKBID	NFKB inhibitor delta	NF-kappaB binding	1.183	1.228	1.072
PIAS3	protein inhibitor of activated STAT 3	Zinc ion binding; protein N-terminus binding; enzyme binding; protein C-terminus binding; nucleic acid binding; SUMO ligase activity; potassium channel regulator activity	1.082	1.116	1.008
ZYX	Zyxin	Zinc ion binding	1.076	1.139	1.055
IL1A	Interleukin 1 alpha	Interleukin-1 receptor binding; protein binding; copper ion binding; cytokine activity	0.932	1.007	0.768
BRD4	Bromodomain containing 4	P53 binding; protein binding; histone acetyl-lysine binding; chromatin binding	0.923	1.002	0.934
PRKCA	Protein kinase C alpha	Metal ion binding; protein binding; zinc ion binding; ATP binding; calcium-dependent protein kinase C activity	0.886	1.173	0.903
NOTCH1	Notch 1	RNA polymerase II transcription factor binding transcription factor activity involved in positive regulation of transcription; sequence-specific DNA binding; calcium ion binding; enzyme inhibitor activity	0.841	0.894	0.699
SEMA7A	Semaphorin 7A (John Milton Hagen blood group)	Integrin binding; receptor activity	0.763	0.806	0.743
NISCH	Nischarin	Phosphatidylinositol binding; G-protein coupled amine receptor activity; integrin binding	0.742	0.869	0.796
FFAR4	Free fatty acid receptor 4	Taste receptor activity; G-protein coupled receptor activity; fatty acid binding	0.675	0.675	0.613
RORA	RAR related orphan receptor A		0.664	0.830	0.766
PRKCD	Protein kinase C delta	Insulin receptor substrate binding; protein kinase C activity; protein C-terminus binding; metal ion binding; nonmembrane spanning protein tyrosine kinase activity	0.638	0.822	0.625
IL15	Interleukin 15	Cytokine activity	0.628	0.646	0.623
MEIS2	Meis homeobox 2	Sequence-specific DNA binding transcription factor activity; corepressor activity	0.625	0.688	0.679
CREB3L1	Camp responsive element binding protein 3 like 1	Chromatin binding; sequence-specific DNA binding transcription factor activity; RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription; sequence-specific DNA binding	0.550	0.575	0.467
SLC26A2	Solute carrier family 26 member 2	Secondary active sulfate transmembrane transporter activity	0.457	0.618	0.593
TYRO3	TYRO3 protein tyrosine kinase	ATP binding; protein heterodimerization activity; receptor signaling protein tyrosine kinase activity; ATP binding	0.430	0.620	0.576
IKBKB	Inhibitor of nuclear factor kappa B kinase subunit beta	Protein kinase activity; ATP binding; scaffold protein binding; I kappa B kinase activity; protein heterodimerization activity; serine/threonine kinase activity	0.410	0.500	0.470
IL1RAP	Interleukin 1 receptor accessory protein	Interleukin-1 receptor activity; interleukin-1 receptor activity; signal transducer activity	0.385	0.448	0.374
TNFAIP3	TNF alpha induced protein 3	Ubiquitin binding; DNA binding; ligase activity; kinase binding; ubiquitin thiolesterase activity; protein self-association; ubiquitin-specific protease activity; zinc ion binding	0.377	0.451	0.434

Contd...

Table 3: Contd...

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated Cells	RPKM value after EM treatment on LPS treated Cells
ISL1	ISL LIM homeobox 1	Zinc ion binding; estrogen receptor binding; sequence-specific DNA binding transcription factor activity; RNA polymerase II activating transcription factor binding; ligand-dependent nuclear receptor binding; enhancer sequence-specific DNA binding; chromatin binding; bHLH transcription factor binding	0.364	0.390	0.336
CD14	CD14 molecule	Lipoteichoic acid binding; peptidoglycan receptor activity; opsonin receptor activity; lipopolysaccharide binding	0.349	0.378	0.200
UCN	Urocortin	Corticotropin-releasing hormone receptor 1 binding; histone deacetylase inhibitor activity; neuropeptide hormone activity	0.322	0.783	0.770
RASGRP1	RAS guanyl releasing protein 1	Calcium ion binding; guanyl-nucleotide exchange factor activity	0.294	0.611	0.427
PXK	PX domain containing serine/threonine kinase like	Protein kinase activity; ATP binding; protein C-terminus binding; actin binding; transferase activity, actin binding; protein kinase activity; nucleotide binding; phosphatidylinositol binding	0.234	0.251	0.246
CCL4	C-C motif chemokine ligand 4	Identical protein binding; cytokine activity; chemokine activity; CCR5 chemokine receptor binding; CCR1 chemokine receptor binding	0.227	0.244	0.181
CSF1R	Colony stimulating factor 1 receptor	Transmembrane receptor protein tyrosine kinase activity; macrophage colony-stimulating factor receptor activity	0.212	0.240	0.210
NLRC4	NLR family CARD domain containing 4	Magnesium ion binding ATP binding; identical protein binding; protein homodimerization activity	0.207	0.344	0.290

Top 50 genes with greatest increase in RPKM value caused by LPS treatment and that were reversely directed (downregulated) after treatment with 100 Hz f-PEMF for 30 seconds. RPKM=Read Per Kilobase Million, LPS=Lipopolysaccharide, PEMF=Pulsed electro-magnetic frequency, ATP=Adenosine Tri-Phosphate, MAP=Mitogen activated protein, SMAD=Proteins homologous to the *Caenorhabditis elegans* SMA ("small" worm phenotype) and *Drosophila* MAD ("Mothers Against Decapentaplegic") family of genes, SUMO=small ubiquitin-related modifier proteins.

similar to the analysis of inflammation-associated genes described above, by using the same CLC software, we filtered a panel of toxicology associated genes from the entire list of 60,446 annotated genes used for our RNAseq analysis. We then examined the influence of focused PEMF on the expression regulation of this toxicology associated panel genes. We again sub-divided the toxicology gene panel by two groups i) those of which the expression levels were increased by LPS and ii) those of which were decreased by LPS stimulation in adult human intestinal primary epithelial cells. As can be seen in Table 5 and 6, consistent with the observation in inflammatory genes panel, the expression level of most of the toxicology genes that were either upregulated [Table 5] or downregulated [Table 6] by LPS were put into the reverse trend by focused PEMF treatment. Although this was a preliminary study with one single dose of 100 Hz EM, these results provide important evidence that exposure to the low frequency of PEMF may play a role in the restoration of physiologic homeostasis without any overt toxic effect. Of course, further studies are necessary to characterize the safety and efficacy ranges in biological cell, tissue, organ, and/or system-specific manner.

Discussion

Inflammation is considered to be the underlying cause of most of the major chronic organ system clinical diseases.^[10,35] For example, pathophysiological processes of cancer, heart attack, Alzheimer, arthritis, and many other chronic conditions are associated with the initiation and perpetuation of inflammation in the respective tissues or organs.^[36-39] Such chronic diseases involve multiple structural and functional alterations within the inner core of cells.^[40-45] All of these alterations are fundamentally associated with changes in gene expression profile, which in turn, impair physiological properties in specific cells, tissues, and organs in the human body. For many of these diseases, currently, no adequately safe treatment exists.

In the present study, we examined the effect of a single exposure of 100 Hz on gene expression regulation in LPS stimulated human intestinal epithelial cells. The adult human GI stem cell-like primary epithelial cells used in our study serves as an interesting model to study inflammatory responses as well as to study the biological effects of the EM field because there

Table 4: Pulsed electro-magnetic field treatment reverses downregulated expression of inflammation-associated genes in LPS-stimulated human gastrointestinal epithelial cells

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated cells	RPKM value After EM treatment on LPS treated cells
RPS19	Ribosomal protein S19	Structural constituent of ribosome; protein kinase binding; fibroblast growth factor binding; protein homodimerization activity	161.125	129.136	160.552
SPINK1	Serine peptidase inhibitor, Kazal type 1	Endopeptidase inhibitor activity; serine-type endopeptidase inhibitor activity	116.957	91.707	110.754
GSTP1	Glutathione S-transferase pi 1	Kinase regulator activity; dinitrosyl-iron complex binding; S-nitrosoglutathione binding; JUN kinase binding; nitric oxide binding	95.039	76.287	114.181
HMOX1	Heme oxygenase 1	Heme binding; heme oxygenase enzyme binding; metal ion binding; phospholipase D activity; signal transducer activity	83.066	59.170	83.649
PSMB4	Proteasome subunit beta 4	Lipopolysaccharide binding; threonine-type endopeptidase activity	62.624	52.769	78.850
LYZ	Lysozyme	Identical protein binding; lysozyme activity; lysozyme activity	41.304	36.018	56.074
PRDX5	Peroxiredoxin 5	Protein dimerization activity; peroxiredoxin activity; cysteine-type endopeptidase inhibitor activity involved in apoptotic process; antioxidant activity; peroxynitrite reductase activity	39.968	30.373	41.129
GPX2	Glutathione peroxidase 2	Glutathione peroxidase activity; electron carrier activity	34.391	28.377	36.416
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	Dopachrome isomerase activity; cytokine receptor binding; chemoattractant activity; phenylpyruvate tautomerase activity	32.053	27.165	43.281
PSMA6	Proteasome subunit alpha 6	RNA binding; endopeptidase activity; NF-kappaB binding; purine ribonucleoside triphosphate binding; threonine-type endopeptidase activity	28.433	23.160	35.133
IER3	Immediate early response 3	Protein binding	26.756	21.269	23.137
GPX4	Glutathione peroxidase 4	Selenium binding; phospholipid-hydroperoxide glutathione peroxidase activity	18.773	12.464	16.161
KDELR1	KDEL endoplasmic reticulum protein retention receptor 1	KDEL sequence binding; ER retention	15.871	14.708	18.319
PARK7	Parkinsonism associated deglycase	Peptidase activity; scaffold protein binding; androgen receptor binding; transcription factor binding; identical protein binding; cytokine binding; small protein activating enzyme binding	15.545	12.262	15.310
HIF1A	Hypoxia inducible factor 1 alpha subunit	RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity; Hsp90 protein binding; nuclear hormone receptor binding; sequence-specific DNA binding; histone deacetylase binding; signal transducer activity; protein heterodimerization activity	14.022	12.916	15.088
RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	GTP binding; thioesterase binding; Rho GDP-dissociation inhibitor binding; protein binding; GTP-dependent protein binding; protein binding; GTPase activity; protein kinase binding; Rab GTPase binding	13.307	12.125	14.683
VIMP	Selenoprotein S	Selenium binding; antioxidant activity	13.267	11.834	14.476
PPP1CA	Protein phosphatase 1 catalytic subunit alpha	Ribonucleoprotein complex binding; phosphatase activity; metal ion binding; hydrolase activity; protein serine/threonine phosphatase activity	13.040	9.901	15.339
ELF3	E74 like ETS transcription factor 3	Transcription coactivator activity; sequence-specific DNA binding; RNA polymerase II transcription factor activity	12.861	9.434	12.026

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Table 4: Contd...

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated cells	RPKM value After EM treatment on LPS treated cells
NFE2L1	Nuclear factor, erythroid 2 like 1	DNA binding; sequence-specific DNA binding transcription factor activity; transcription cofactor activity; RNA polymerase II transcription factor binding transcription factor activity involved in positive regulation of transcription	11.932	10.409	11.306
GPX1	Glutathione peroxidase 1	Selenium binding; SH3 domain binding; SH3 domain binding; glutathione peroxidase activity; phospholipid-hydroperoxide glutathione peroxidase activity	11.143	8.076	11.803
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	Transcription regulatory region DNA binding; sequence-specific DNA binding transcription factor activity; R-SMAD binding	10.843	9.031	9.100
PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1	Protein binding	10.049	9.071	9.707
ZFP36	ZFP36 ring finger protein	Protein kinase binding; DNA binding; mRNA 3'-UTR AU-rich region binding; metal ion binding; C-C chemokine binding; single-stranded RNA binding; 14-3-3 protein binding; AU-rich element binding	9.985	8.428	10.301
HMGB1	High mobility group box 1	Repressing transcription factor binding; damaged DNA binding; double-stranded DNA binding; transcription factor binding; sequence-specific DNA binding transcription factor activity; chemoattractant activity; RAGE receptor binding	9.881	8.345	11.695
TBC1D23	TBC1 domain family member 23	Rab GTPase activator activity	9.455	9.294	10.348
TBK1	TANK binding kinase 1	ATP binding; protein serine/threonine kinase activity; phosphoprotein binding	9.395	8.656	8.944
ABCF1	ATP binding cassette subfamily F member 1	ATP binding; ribosome binding; translation activator activity; ATPase activity; nucleic acid binding	9.339	8.066	9.286
CASP7	Caspase 7	Aspartic-type endopeptidase activity; cysteine-type endopeptidase activity involved in apoptotic process	9.217	8.812	11.466
UQCRC1	Ubiquinol-cytochrome c reductase core protein I	Protein complex binding; metal ion binding; ubiquinol-cytochrome-c reductase activity	9.135	8.053	11.470
YIPF5	Yip1 domain family member 5		8.244	8.243	8.348
CYBA	Cytochrome b-245 alpha chain	Electron carrier activity; SH3 domain binding; superoxide-generating NADPH oxidase activity; protein heterodimerization activity heme binding	7.169	5.067	10.042
ETS2	ETS proto-oncogene 2, transcription factor	Sequence-specific DNA binding RNA polymerase II transcription factor activity; RNA polymerase II core promoter proximal region sequence-specific DNA binding; glucocorticoid receptor binding	6.116	5.857	7.556
CASP4	Caspase 4	Cysteine-type endopeptidase activity	6.002	5.071	7.010
AFG3L2	AFG3 like matrix AAA peptidase subunit 2	Metalloendopeptidase activity; unfolded protein binding; ATP binding; zinc ion binding; nucleoside-triphosphatase activity	5.972	5.773	8.393
AIMP1	Aminoacyl tRNA synthetase complex interacting multifunctional protein 1	Protein homodimerization activity; tRNA binding; cytokine activity	5.907	5.187	7.162

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Table 4: Contd...

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated cells	RPKM value After EM treatment on LPS treated cells
HSD17B4	Hydroxysteroid 17-beta dehydrogenase 4	3-hydroxyacyl-CoA dehydrogenase activity; 3alpha, 7alpha, 12alpha-trihydroxy-5beta-cholest-24-enoyl-CoA hydratase activity; 17-beta-hydroxysteroid dehydrogenase; receptor binding; oxidoreductase activity; long-chain-enoyl-CoA hydratase activity; isomerase activity	5.833	5.545	5.938
STK39	Serine/threonine kinase 39	ATP binding; receptor signaling protein serine/threonine kinase activity	5.725	5.254	5.862
PSMG2	Proteasome assembly chaperone 2	Protein binding	5.479	5.135	7.233
TXNIP	Thioredoxin interacting protein	Enzyme inhibitor activity; ubiquitin protein ligase binding	5.329	5.036	8.812
PPARG	Peroxisome proliferator activated receptor gamma	Drug binding; ligand-dependent nuclear receptor transcription coactivator activity; sequence-specific DNA binding transcription factor activity; arachidonic acid binding; steroid hormone receptor activity; ligand-activated sequence-specific DNA binding RNA polymerase II transcription factor activity; retinoid X receptor binding	5.036	4.435	5.485
EXT2	Exostosin glycosyltransferase 2	N-acetylglucosaminyl-proteoglycan 4-beta-glucuronosyltransferase activity	4.840	4.550	6.171
PEX16	peroxisomal biogenesis factor	Protein C-terminus binding	4.666	3.473	5.765
TNIP2	TNFAIP3 interacting protein 2	Polyubiquitin binding; protein kinase binding	4.666	4.393	4.709
BAX	BCL2 associated X, apoptosis regulator	BH3 domain binding; identical protein binding; channel activity; protein homodimerization activity	4.570	3.649	4.905
RIPK2	receptor interacting serine/threonine kinase 2	LIM domain binding; CARD domain binding; transferase activity, transferring phosphorus-containing groups; non-membrane spanning protein tyrosine kinase activity; ATP binding	4.333	3.972	5.065
TNIP1	TNFAIP3 interacting protein 1	Mitogen-activated protein kinase binding; ubiquitin-specific protease activity	4.024	3.389	4.704
TNFRSF1A	TNF receptor superfamily member 1A	Tumor necrosis factor-activated receptor activity	3.935	3.044	4.533
CAT	Catalase	NADP binding; oxidoreductase activity, acting on peroxide as acceptor; catalase activity	3.339	2.877	3.166
TUSC2	Tumor suppressor candidate 2		3.310	2.446	3.048

Top 50 genes with greatest decrease in RPKM value caused by LPS treatment and that were reversely directed (upregulated) after treatment with 100 Hz f-PEMF for 30 s. RPKM=Read Per Kilobase Million, LPS=Lipopolysaccharide, PEMF=Pulsed electro-magnetic frequency, LIM=Three proteins (Lin-11, Isl-1, Mec-3) involved in cytoskeletal organization, CARD=Caspase recruitment domain proteins, NADP=nicotinamide adenine dinucleotide.

exists a large body of information in the literature for their interactions with bacterial toxins as well as transmission of electromagnetically coupled energy through these cells. Bacterial endotoxin induces a potent inflammatory response by these cells involving a variety of mechanisms.^[46,47] Previous studies have shown in the past that LPS induces proinflammatory cytokine production as well modulates surface antigen expression in these cells.^[48,49] EM transmission is the basic principle used in many clinical investigational procedures. Visualization of the epithelial surface inside the GI tract by battery-less wireless capsule endoscopy is based on

EM transmission. Thus, these cells suited us well for our experimental design.

Not surprisingly, our experimental results demonstrated that LPS caused massive alterations in the human genome profile both in expression and transcription levels. An interesting phenomenon was observed that the number of genes that were turned on (*de novo* induced) by LPS (1 µg/ml) stimulation was almost equal to the number of genes that were silenced during the inflammatory response to LPS by these cells [Tables 1 and 2]. More interestingly, our experimental

Table 5: Pulsed electro-magnetic field treatment reverses upregulated expression of toxicology pathway associated genes in LPS-Stimulated human gastrointestinal epithelial cells

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated cells	RPKM value After EM treatment on LPS treated cells
WAPAL	WAPL cohesin release factor	Protein binding	8.211	9.780	9.629
RPS6KB1	Ribosomal protein S6 kinase B1	Protein serine/threonine/tyrosine kinase activity; ATP binding	2.898	3.135	2.752
TMEM181	Transmembrane protein 181	Toxic substance binding	1.395	1.999	1.756
BLMH	Bleomycin hydrolase	Carboxypeptidase activity; cysteine-type endopeptidase activity; aminopeptidase activity	1.319	1.685	1.584
ERCC6	ERCC excision repair 6, chromatin remodeling factor	Chromatin binding; DNA binding; ATP binding; helicase activity; DNA-dependent ATPase activity; protein C-terminus binding	1.022	1.388	1.111
HTR1D	5-hydroxytryptamine receptor 1D	Serotonin receptor activity; serotonin binding	0.286	0.305	0.263
CCL4	C-C motif chemokine ligand 4	CCR5 chemokine receptor binding; CCR1 chemokine receptor binding	0.227	0.244	0.181
SLC30A4	Solute carrier family 30 member 4	Zinc ion transmembrane transporter activity	0.194	0.311	0.225
PDZD3	PDZ domain containing 3	Guanylate cyclase inhibitor activity; protein C-terminus binding; protein C-terminus binding; protein binding; protein binding; ion channel inhibitor activity	0.103	0.142	0.083
SLC6A4	Solute carrier family 6 member 4	Monoamine transmembrane transporter activity; cocaine binding; serotonin: sodium symporter activity; actin filament binding; syntaxin-1 binding; Rab GTPase binding	0.092	0.112	0.077
SLC7A8	Solute carrier family 7 member 8	Amino acid transmembrane transporter activity; organic cation transmembrane transporter activity; toxin transporter activity; L-amino acid transmembrane transporter activity	0.090	0.104	0.077
SCN9A	Sodium voltage-gated channel alpha subunit 9	Voltage-gated sodium channel activity	0.054	0.078	0.031
INMT	Indolethylamine N-methyltransferase	Amine N-methyltransferase activity; thioether S-methyltransferase activity	0.053	0.071	0.057
TTPA	Alpha tocopherol transfer protein	Transporter activity; phosphatidylinositol-3,4-bisphosphate binding; Vitamin E binding	0.039	0.050	0.009
CES1	Carboxylesterase 1	Methylumbelliferyl-acetate deacetylase activity; carboxylic ester hydrolase activity	0.035	0.058	0.035
TRPM6	Transient receptor potential cation channel subfamily M member 6	Protein serine/threonine kinase activity; ATP binding; metal ion binding; calcium channel activity	0.033	0.044	0.031
CPS1	Carbamoyl-phosphate synthase 1	Carbamoyl-phosphate synthase; glutamate binding; calcium ion binding; ATP binding; phospholipid binding; endopeptidase activity; carbamoyl-phosphate synthase ;modified amino acid binding	0.024	0.031	0.007
DSG1	Desmoglein 1 [Calcium ion binding; toxic substance binding; gamma-catenin binding	0.021	0.029	0.014

RPKM value of 18 toxicological function associated genes (filtered by CLC software) that were constitutively expressed (4th column) were upregulated by LPS treatment (5th column) and that were reversely directed (downregulated) (6th column) after treatment with 100 Hz f-PEMF for 30 s. RPKM=Read Per Kilobase Million, LPS=Lipopolysaccharide, PEMF=Pulsed electro-magnetic frequency, CLC=Software developed by CLC-bio, ATP=Adenosine Tri-Phosphate

results demonstrated that a single treatment of low frequency focused PEMF (100 Hz) for 30s reverse directed the LPS actions on gene expression regulation. A large panel of inflammation-associated genes that were upregulated by LPS stimulation was downregulated by PEMF treatment and vice versa [Table 3 and 4]. We

expect to identify valuable information from this simple and single pilot study of human genome expression regulation conducted in that may guide for drawing new strategies for discovery and development of new, safer, more effective, and economical treatments for chronic inflammatory diseases. In this regard, we highlight one

Table 6: Pulsed electro-magnetic frequency treatment reverses downregulated expression of toxicology pathway associated genes in lipopolysaccharide -stimulated human gastrointestinal epithelial cells

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated cells	RPKM value after EM treatment on LPS treated cells
NQO1	NAD (P) H quinone dehydrogenase 1	NAD; protein binding; superoxide dismutase activity; cytochrome-b5 reductase activity, acting on NAD;	38.223	33.218	48.694
PTGR1	Prostaglandin reductase 1	13-prostaglandin reductase activity; oxidoreductase activity; zinc ion binding; 2-alkenal reductase	25.064	20.958	20.972
CDKN1A	Cyclin dependent kinase inhibitor 1A	Cyclin-dependent protein serine/threonine kinase inhibitor activity	17.952	14.931	16.567
ASNS	Asparagine synthetase (glutamine-hydrolyzing)	Asparagine synthase; cofactor binding; ATP binding	12.576	11.823	12.052
GPX1	Glutathione peroxidase 1	Selenium binding; SH3 domain binding; glutathione peroxidase activity; phospholipid-hydroperoxide glutathione peroxidase activity	11.143	8.076	11.803
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	Transcription regulatory region DNA binding; sequence-specific DNA binding transcription factor activity; R-SMAD binding; DNA binding; transcription factor binding	10.843	9.031	9.100
CDK4	Cyclin dependent kinase 4	ATP binding; cyclin-dependent protein serine/threonine kinase activity	9.391	8.565	10.934
EPHX1	Epoxide hydrolase 1 [Source: HGNC Symbol; Acc: HGNC: 3401]	Cis-stilbene-oxide hydrolase activity; epoxide hydrolase activity	8.499	7.511	10.208
BAX	BCL2 associated X, apoptosis regulator	BH3 domain binding; protein binding; identical protein binding; protein heterodimerization activity	4.570	3.649	4.905
TK1	Thymidine kinase 1	Nucleoside kinase activity; identical protein binding; ATP binding; thymidine kinase activity; ATP binding; zinc ion binding	4.312	3.313	6.253
SCFD1	Sec1 family domain containing 1	Syntaxin binding; protein N-terminus binding	3.987	3.851	3.853
PINK1	PTEN induced putative kinase 1	Kinase activity; calcium-dependent protein kinase activity; ubiquitin protein ligase binding; ATP binding; magnesium ion binding; C3HC4-type RING finger domain binding; protein serine/threonine kinase activity	3.869	3.779	3.820
PHAX	Phosphorylated adaptor for RNA export	Toxic substance binding; RNA binding	2.350	2.318	3.268
CDK1	Cyclin dependent kinase 1	Cyclin-dependent protein serine/threonine kinase activity; protein binding; RNA polymerase II carboxy-terminal domain kinase activity	1.687	1.548	2.216
CNP	2',3'-cyclic nucleotide 3' phosphodiesterase	2',3'-cyclic-nucleotide 3'-phosphodiesterase activity; cyclic nucleotide binding; RNA binding	1.383	1.238	1.429
MGMT	O-6-methylguanine-DNA methyltransferase	Methylated-DNA-[protein]-cysteine S-methyltransferase activity; methyltransferase activity; DNA binding; calcium ion binding	0.996	0.892	1.150
HDAC6	Histone deacetylase 6	NAD-dependent histone deacetylase activity; hydrolase activity; microtubule binding; deacetylase activity; histone deacetylase activity; Hsp90 protein binding	0.787	0.751	0.869
TYMS	Thymidylate synthetase	Cofactor binding; protein homodimerization activity; drug binding; folic acid binding; mRNA binding; nucleotide binding; thymidylate synthase activity	0.767	0.675	1.061
CYP1B1	Cytochrome P450 family 1 subfamily B member 1	Monoxygenase activity; aromatase activity; heme binding; oxygen binding; iron ion binding; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	0.650	0.501	0.639
BDH1	3-hydroxybutyrate dehydrogenase 1	3-hydroxybutyrate dehydrogenase activity; oxidoreductase activity; 3-hydroxybutyrate dehydrogenase activity; phospholipid binding	0.547	0.533	1.004
BPHL	Biphenyl hydrolase like	Hydrolase activity	0.422	0.417	0.492

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Table 6: Contd...

Symbol	Gene name	Gene function	RPKM value in untreated cells	RPKM value in LPS treated cells	RPKM value after EM treatment on LPS treated cells
DHRS2	dehydrogenase/reductase 2 [Carbonyl reductase; oxidoreductase activity	0.314	0.235	0.325
FAS	Fas cell surface death receptor	Identical protein binding; kinase binding; transmembrane signaling receptor activity	0.236	0.229	0.379
SLC23A1	Solute carrier family 23 member 1	Transporter activity; L-ascorbic acid transporter activity; L-ascorbic acid transporter activity; dehydroascorbic acid transporter activity; L-ascorbate: sodium symporter activity; nucleobase transmembrane transporter activity	0.093	0.082	0.127
PON1	Paraoxonase 1	Phospholipid binding; arylesterase activity; calcium ion binding; arylalkylphosphatase activity; protein homodimerization activity	0.090	0.035	0.050

RPKM value of 25 toxicological function associated genes (filtered by CLC software) that were constitutively expressed (4th column) were downregulated by LPS treatment (5th column) and that were reversely directed (upregulated) (6th column) after treatment with 100 Hz f-PEMF for 30 s. RPKM=Read Per Kilobase Million, LPS=Lipopolysaccharide, PEMF=Pulsed electro-magnetic field, CLC=Software developed by CLC-bio

critical observation that an exposure of focused PEMF (100 Hz for 30 s) inhibits prostaglandin-endoperoxide synthase (PTGS2) by ~ 40% [Table 3]. PTGS2 is known as cyclooxygenase-2 (Cox2), which is the key enzyme in the prostaglandin biosynthesis pathway and as is normally targeted by NSAID. It is worth mentioning that NSAIDs represent one of the most widely prescribed drugs for treatments of inflammation, pain, and fever. Their effects are largely attributed to the suppression of prostaglandin production by inhibiting cyclooxygenases. However, NSAIDs are also known to cause huge health burdens when taken for longer periods. Chronic use of NSAIDs causes GI ulcers, hemorrhage, liver toxicity, cardiovascular conditions, and many adverse effects in human health. Therefore in addition to general homeostatic restoration, focused PEMF may offer a solution for safety and efficacy concerns of currently used anti-inflammatory drugs. Finally, our analysis on toxicological panel shows a similar trend of reversal of the LPS effect without any overt induction of toxicological genes [Table 5 and 6].

Taken together our results provide new insights toward the understanding of potential mechanisms by which focused PEMF offering effects on subtlest constituents (genes) of the human body. A single exposure of 100 Hz f-PEMF for 30s to LPS stimulated cells resulted in reverse regulation of gene expression profile. The expression of genes that were increased by a known immune-stimulatory stimuli LPS were in most cases, reversed either by complete restoration or by bringing closure to the level of that was seen in the unstimulated (baseline control) cells. In some cases, focused PEMF downregulated the gene expression even below the constitutive level. For example, the PTGS2 gene was suppressed by 40% (from a RPKM value of. 143 in LPS treated cells to RPKM value of 3.579 in LPS + f-PEMF treated cells) by a low-frequency f-PEMF for the minuscule amount of time (30s) opens

a new possibility for filling the safety and efficacy gaps in current therapeutic approaches for the treatment of chronic diseases.

In conclusion, alterations in epigenetic homeostasis that occur in various acute and chronic health conditions' resulting symptoms, syndromes, pain, and suffering might be associated with a dysregulated EM energy field within the cells of the affected tissues, organs, or organ systems. A reversal of such EM energy dysbalance might be possible through the creation of an optimum EM field within the damaged body parts. The main limitation of this study that it is of exploratory nature with a limited number of experiments. Therefore, our data need to be confirmed in larger studies with additional controls and more detailed comparative analysis. Further studies of EM therapy mechanisms and dosimetry for specific health conditions with devices such as the one used in our study may contribute significantly in the reduction of both health-and economic burdens caused by many chronic health conditions for which modern medicine offers no solution.

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Conflicts of interest

There are no conflicts of interest.

References

- Allen RJ, Porte J, Braybrooke R, Flores C, Fingerlin TE, Oldham JM, *et al.* Genetic variants associated with susceptibility to idiopathic pulmonary fibrosis in people of European ancestry: A genome-wide association study. *Lancet Respir Med* 2017;5:869-80.
- David T, Ling SF, Barton A. Genetics of immune-mediated inflammatory diseases. *Clin Exp Immunol* 2018;193:3-12.
- Kerstein A, Schüler S, Cabral-Marques O, Fazio J, Häsler R, Müller A, *et al.* Environmental factor and inflammation-driven

- alteration of the total peripheral T-cell compartment in granulomatosis with polyangiitis. *J Autoimmun* 2017;78:79-91.
4. Kunnumakkara AB, Sailo BL, Banik K, Harsha C, Prasad S, Gupta SC, *et al.* Chronic diseases, inflammation, and spices: How are they linked? *J Transl Med* 2018;16:14.
 5. Smith JA, Zhao W, Wang X, Ratliff SM, Mukherjee B, Kardias SL, *et al.* Neighborhood characteristics influence DNA methylation of genes involved in stress response and inflammation: The multi-ethnic study of atherosclerosis. *Epigenetics* 2017;12:662-73.
 6. Langgartner D, Palmer A, Rittlinger A, Reber SO, Huber-Lang M. Effects of prior psychosocial trauma on subsequent immune response after experimental thorax trauma. *Shock* 2018;49:690-7.
 7. Ritzel RM, Doran SJ, Barrett JP, Henry RJ, Ma EL, Faden AI, *et al.* Chronic alterations in systemic immune function after traumatic brain injury. *J Neurotrauma* 2018;35:1419-36.
 8. Kezić A, Stajic N, Thaiss F. Innate immune response in kidney ischemia/reperfusion injury: Potential target for therapy. *J Immunol Res* 2017;2017:6305439.
 9. Ravichandran S, Michelucci A, Del Sol A. Integrative computational network analysis reveals site-specific mediators of inflammation in Alzheimer's disease. *Front Physiol* 2018;9:154.
 10. Egawa M, Mitamura Y, Akaiwa K, Semba K, Kinoshita T, Uchino E, *et al.* Changes of choroidal structure after corticosteroid treatment in eyes with Vogt-Koyanagi-Harada disease. *Br J Ophthalmol* 2016;100:1646-50.
 11. Winkelman C, Higgins PA, Chen YJ, Levine AD. Cytokines in chronically critically ill patients after activity and rest. *Biol Res Nurs* 2007;8:261-71.
 12. Nagahisa A, Asai R, Kanai Y, Murase A, Tsuchiya-Nakagaki M, Nakagaki T, *et al.* Non-specific activity of (+/-)-CP-96,345 in models of pain and inflammation. *Br J Pharmacol* 1992;107:273-5.
 13. Joshi P, Dhaneshwar SS. An update on disease modifying antirheumatic drugs. *Inflamm Allergy Drug Targets* 2014;13:249-61.
 14. Cudaback E, Jorstad NL, Yang Y, Montine TJ, Keene CD. Therapeutic implications of the prostaglandin pathway in Alzheimer's disease. *Biochem Pharmacol* 2014;88:565-72.
 15. Bolay H, Durham P. Pharmacology. *Handb Clin Neurol* 2010;97:47-71.
 16. Aghazadeh-Habashi A, Asghar W, Jamali F. Drug-disease interaction: Effect of inflammation and nonsteroidal anti-inflammatory drugs on cytochrome P450 metabolites of arachidonic acid. *J Pharm Sci* 2018;107:756-63.
 17. Chung EY, Tat ST. Nonsteroidal anti-inflammatory drug toxicity in children: A clinical review. *Pediatr Emerg Care* 2016;32:250-3.
 18. de Pouvourville G, Tasch RF. The economic consequences of NSAID-induced gastrointestinal damage. *Eur J Rheumatol Inflamm* 1993;13:33-40.
 19. Trippel SB. The unmet anti-inflammatory needs in orthopedics. *Am J Orthop (Belle Mead NJ)* 1999;28 Suppl 3:3-7.
 20. Pardutz A, Schoenen J. NSAIDs in the acute treatment of migraine: A review of clinical and experimental data. *Pharmaceuticals (Basel)* 2010;3:1966-87.
 21. Hunsche E, Chancellor JV, Bruce N. The burden of arthritis and nonsteroidal anti-inflammatory treatment. A European literature review. *Pharmacoeconomics* 2001;19 Suppl 1:1-15.
 22. Iwasa K, Reddi AH. Pulsed electromagnetic fields and tissue engineering of the joints. *Tissue Eng Part B Rev* 2018;24:144-54.
 23. Aragona SE, Mereghetti G, Lotti J, Vosa A, Lotti T, Canavesi E. Electromagnetic field in control tissue regeneration, pelvic pain, neuro-inflammation and modulation of non-neuronal cells. *J Biol Regul Homeost Agents* 2017;31 2 Suppl 2:219-25.
 24. Bilgin HM, Celik F, Gem M, Akpolat V, Yildiz I, Ekinci A, *et al.* Effects of local vibration and pulsed electromagnetic field on bone fracture: A comparative study. *Bioelectromagnetics* 2017;38:339-48.
 25. Ongaro A, Pellati A, Bagheri L, Fortini C, Setti S, De Mattei M. Pulsed electromagnetic fields stimulate osteogenic differentiation in human bone marrow and adipose tissue derived mesenchymal stem cells. *Bioelectromagnetics* 2014;35:426-36.
 26. Ferroni L, Tocco I, De Pieri A, Menarin M, Fermi E, Piattelli A, *et al.* Pulsed magnetic therapy increases osteogenic differentiation of mesenchymal stem cells only if they are pre-committed. *Life Sci* 2016;152:44-51.
 27. Jiao M, Lou L, Jiao L, Hu J, Zhang P, Wang Z, *et al.* Effects of low-frequency pulsed electromagnetic fields on plateau frostbite healing in rats. *Wound Repair Regen* 2016;24:1015-22.
 28. Panja A. A novel method for the establishment of a pure population of nontransformed human intestinal primary epithelial cell (HIPEC) lines in long term culture. *Lab Invest* 2000;80:1473-5.
 29. Martin CA, Panja A. Cytokine regulation of human intestinal primary epithelial cell susceptibility to Fas-mediated apoptosis. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G92-104.
 30. Kim YO, Han SB, Lee HW, Ahn HJ, Yoon YD, Jung JK, *et al.* Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of *Inonotus obliquus*. *Life Sci* 2005;77:2438-56.
 31. Haller D, Bode C, Hammes WP. Cytokine secretion by stimulated monocytes depends on the growth phase and heat treatment of bacteria: A comparative study between lactic acid bacteria and invasive pathogens. *Microbiol Immunol* 1999;43:925-35.
 32. Pardon MC. Lipopolysaccharide hyporesponsiveness: Protective or damaging response to the brain? *Rom J Morphol Embryol* 2015;56:903-13.
 33. Zielen S, Trischler J, Schubert R. Lipopolysaccharide challenge: Immunological effects and safety in humans. *Expert Rev Clin Immunol* 2015;11:409-18.
 34. Voss OH, Murakami Y, Pena MY, Lee HN, Tian L, Margulies DH, *et al.* Lipopolysaccharide-induced CD300b receptor binding to toll-like receptor 4 alters signaling to drive cytokine responses that enhance septic shock. *Immunity* 2016;44:1365-78.
 35. Umeno J, Esaki M, Hirano A, Fuyuno Y, Ohmiya N, Yasukawa S, *et al.* Clinical features of chronic enteropathy associated with SLCO2A1 gene: A new entity clinically distinct from Crohn's disease. *J Gastroenterol* 2018;53:907-15.
 36. Kallwellis-Opara A, Dörner A, Poller WC, Noutsias M, Kühl U, Schultheiss HP, *et al.* Autoimmunological features in inflammatory cardiomyopathy. *Clin Res Cardiol* 2007;96:469-80.
 37. Stephenson J, Nutma E, van der Valk P, Amor S. Inflammation in CNS neurodegenerative diseases. *Immunology* 2018;154:204-19.
 38. Dalle S, Rossmeislova L, Kopko K. The role of inflammation in age-related sarcopenia. *Front Physiol* 2017;8:1045.
 39. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *Br J Pharmacol* 2006;147 Suppl 1:S232-40.
 40. Gea J, Orozco-Levi M, Barreiro E, Ferrer A, Broquetas J. Structural and functional changes in the skeletal muscles of COPD patients: The "compartments" theory. *Monaldi Arch Chest Dis* 2001;56:214-24.
 41. Chung KF, Adcock IM. Pathophysiological mechanisms of asthma. Application of cell and molecular biology techniques. *Mol Biotechnol* 2001;18:213-32.
 42. Bohadana A, Teculescu D, Martinet Y. Mechanisms of chronic airway obstruction in smokers. *Respir Med* 2004;98:139-51.
 43. Ogata T, Shibamura H, Tromp G, Sinha M, Goddard KA, Sakalihan N, *et al.* Genetic analysis of polymorphisms in biologically relevant candidate genes in patients with abdominal aortic aneurysms. *J Vasc Surg* 2005;41:1036-42.
 44. Masser DR, Otalora L, Clark NW, Kinter MT, Elliott MH, Freeman WM. Functional changes in the neural retina occur in the absence of mitochondrial dysfunction in a rodent model of diabetic retinopathy. *J Neurochem* 2017;143:595-608.
 45. Nishida K, Otsu K. Inflammation and metabolic cardiomyopathy. *Cardiovasc Res* 2017;113:389-98.

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46. Rainard P, Cunha P, Gilbert FB. Innate and adaptive immunity synergize to trigger inflammation in the mammary gland. *PLoS One* 2016;11:e0154172.
47. Biswas G, Bilen S, Kono T, Sakai M, Hikima J. Inflammatory immune response by lipopolysaccharide-responsive nucleotide binding oligomerization domain (NOD)-like receptors in the Japanese pufferfish (*Takifugu rubripes*). *Dev Comp Immunol* 2016;55:21-31.
48. Salomão R, Martins PS, Brunialti MK, Fernandes Mda L, Martos LS, Mendes ME, *et al.* TLR signaling pathway in patients with sepsis. *Shock* 2008;30 (Suppl 1):73-7.
49. Stroehrer UH, Jedani KE, Manning PA. Genetic organization of the regions associated with surface polysaccharide synthesis in *Vibrio cholerae* O1, O139 and *Vibrio anguillarum* O1 and O2: A review. *Gene* 1998;223:269-82.