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Assessment of the Effects of Pulsed Electromagnetic Field Exposure on Murine 3T3 Cell Line: An Investigation into Cytotoxicology

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KEYWORDS

Pulsed electromagnetic field, NIH 3T3 cells, cell proliferation, cytotoxicity, Alamar Blue, Regenerative medicine

ABSTRACT:

Introduction: Pulsed electromagnetic field(PEMF) therapy is gaining significant attention in clinical medicine due to its promising regenerative properties and its ability to modulate pain effectively. The precise biological mechanisms by which PEMF influences cellular processes—particularly those related to cell proliferation and cytotoxicity—remain unclear despite its increasing use. To better understand how PEMF affects these cellular activities and to maximize its therapeutic uses, more research is essential.

Objectives: Monitor cellular viability and proliferation in order to assess the cytotoxicological effects of PEMF exposure on the 3T3 murine fibroblast cell line.

Methods: To offer the best nutrition and avoid bacterial contamination, 3T3 fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. For uniform growth conditions in every sample, the cells were planted at a density of 100,000 cells per 60 mm dish. Using a sine wave pattern, experimental groups were exposed to pulsed electromagnetic fields (PEMFs) at a frequency of 27.12 MHz and a power intensity of 73 mW/cm². This exposure was applied for 12 hours each day over five consecutive days. Control groups did not receive PEMF treatment. Daily, cell viability was quantitatively measured with the Alamar Blue assay, and each experimental condition was performed in triplicate for reliability.

Results: PEMF exposure resulted in a consistently slower proliferation rate in 3T3 fibroblast cells compared to the control group- did not receive PEMF. Over time, the number of cells in both groups increased steadily, but the PEMF-treated samples' growth rate stayed steadily lower. This highlights the quantifiable influence of PEMF on the dynamics of fibroblast cell line proliferation over the course of the study period and shows a notable difference in the cell growth patterns between the two groups.

Conclusions: 3T3 fibroblast growth is markedly inhibited by pulsed electromagnetic field therapy. This has ramifications for regenerative medicine and supports the way electromagnetic fields regulate cellular activity. To clarify mechanisms and optimize exposure conditions, more research is required.

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1. Introduction

One non-invasive therapeutic option that is becoming more and more popular in contemporary medicine is pulsed electromagnetic field (PEMF) therapy(1). PEMF therapy was initially created to promote bone healing following a fracture, but it is also used for a variety of tissue repair and pain management conditions, such as musculoskeletal injuries, wound healing, and even neurorehabilitation(2). Despite substantial evidence supporting clinical efficacy, the biological underpinnings that explain PEMF's effects at the cellular and molecular level remain only partially understood(3).

Cellular responses to electromagnetic exposure depend on field frequency, waveform, intensity, exposure duration and biological context in which they are used. Preclinical studies show that electromagnetic fields can change how genes are expressed, how cells talk to each other, how ions move about, and how energy is used (4)(5)(6). PEMF may control inflammation, cell growth, and differentiation through these processes. These are important for healing and regenerating tissue in the area of the temporomandibular joints (TMJs).

Because of its well-characterized nature, quick proliferation, and reactivity to external stimuli, the 3T3 cell line—which is derived from Mus musculus (mouse) embryonic fibroblasts—has been a mainstay in biological research(7). 3T3 fibroblasts serve as a model for fundamental cell biology, cytotoxicology, and cancer research. They offer a useful platform for evaluating how pharmacological and biophysical treatments affect cell viability and proliferation.

Clarifying PEMF's impact on basic cellular functions is crucial because of its potential for clinical translation. Some investigations have indicated stimulatory effects of PEMF on the proliferation and differentiation of osteoblasts(8) and chondrocytes(9), however, findings regarding fibroblasts are less consistent. Moreover, the molecular foundation of any reported modifications—whether attributed to variations in energy metabolism, oxidative signaling, or cell cycle regulation—requires additional clarification.

2. Objectives

This study aimed to examine the cytotoxicological effects of PEMF on murine 3T3 fibroblasts. The primary aim of the study was: To measure daily changes in cell viability using the Alamar Blue assay and ultimately assess whether PEMF exposure at clinically relevant parameters modulates the proliferation of 3T3 cells.

3. Methods

This study employed 3T3 cell lines for assessing the pulsed electromagnetic field(PEMF) for its cytotoxic properties and its effect on cell proliferation. Standard laboratory conditions were used to obtain and maintain mouse 3T3 fibroblast cells. Dulbecco's Modified Eagle Medium (DMEM, high glucose) was used to cultivate the cells. 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin were added as supplements. To ensure ideal growth and metabolic activity, cultures were kept at 37°C in a humidified atmosphere with 5% CO_2. Cells were passaged at sub-confluent density to maintain exponential proliferation and to reduce contact inhibition that might confound outcomes.

For each experimental run, 3T3 cells were seeded at a density of 1×10^5 cells per 60 mm culture dish. This initial plating density was chosen to ensure adequate proliferation room and to allow for meaningful quantification of differences in cell numbers throughout the experiment. Cultures were allowed to adhere overnight prior to the start of experimental interventions. Three independent replicates per group—PEMF and Control—were included in the study design (Figure 1). Quantitative fluorescence was recorded and tabulated for all samples at the end of each exposure period- every 24 hour cycle from day 1 through 5.

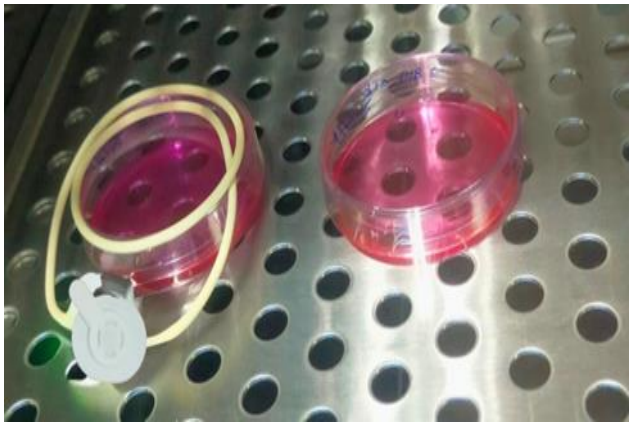

Figure 1- Pulsed electromagnetic field administered to 3T3 cell line. Control remains unexposed.

A pulsed electromagnetic field delivered as a pure sine wave signal with a frequency of 27.12 MHz and an

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intensity of 73 mW/cm² was applied to the intervention group. A specially designed PEMF generator and coil system was used to create the field, which was calibrated and observed to preserve consistency and avoid artifacts like localized heating. In order to simulate a moderate chronic exposure pertinent to biomedical therapy, exposure was carried out for 12 hours every day for five days in a row.

The control group did not receive any PEMF treatment, but they were treated in the same way. To prevent environmental variability, both groups were incubated in the same setting.

The Alamar Blue assay, which was selected for its sensitivity and low cytotoxicity, was used to measure cell viability and proliferation. Aliquots of Alamar Blue reagent were added to each dish at the conclusion of each 24-hour exposure cycle. Following a 2-hour incubation at 37°C to allow metabolic conversion, fluorescence was quantified on a microplate reader (excitation 530 nm, emission 590 nm). The fluorescence intensity was considered proportional to the viable cell number as established in the literature.

Statistical Analysis

The statistical analysis was done using SPSS v26.0(IBM,NY,USA). Descriptive statistical analysis-mean and standard deviation was performed to determine central tendency and variability within each group. To ascertain the significance of the differences between the groups, two-tailed t-tests were used. Plotting line graphs also made it easy to see trends and changes over the course of the experiment. A p-value threshold of less than 0.05 was considered statistically significant, guaranteeing the validity of the study's findings.

4. Results

Baseline Growth and Proliferation

Both groups showed a similar baseline fluorescence at the start of the study, which is a sign of normal cell adhesion and early proliferation. This created a common starting point, facilitating the validity of results obtained by further observation of both PEMF and control groups.

Effects of PEMF Exposure on 3T3 Viability

The fluorescence of the control group increased significantly through Day 4 of the five-day period, indicating ongoing proliferation. On the other hand, PEMF-exposed groups continuously displayed reduced proliferation, becoming more and more different from controls every day (Figure 2 and Figure 3).

Day	Device (Rep 1)	Device (Rep 2)	Device (Rep 3)	Control (Rep 1)	Control (Rep 2)	Control (Rep 3)
1	1,68,743	1,74,389	1,81,934	1,93,603	1,79,572	1,88,935
2	2,88,536	2,91,274	3,02,678	3,69,532	3,57,902	3,61,732
3	4,97,472	5,01,638	5,10,733	6,87,294	6,69,941	6,78,463
4	8,44,055	8,56,985	8,81,604	13,34,183	12,78,852	12,83,404
5	11,32,591	10,92,699	10,92,494	7,86,096	7,79,027	7,75,104

Figure 2- Cell population changes in experimental and control during the course of study.

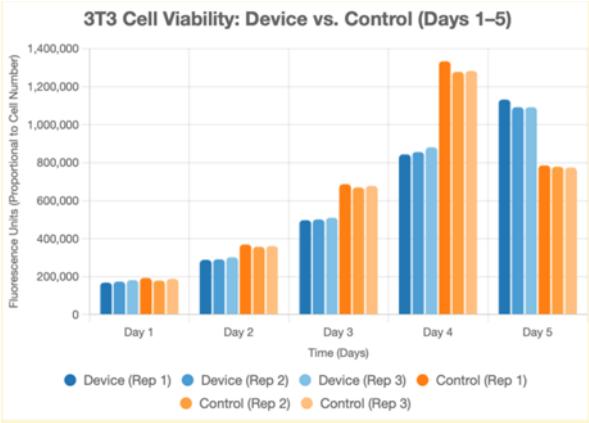

Figure 3- Graphical plot of cell population changes during study. Note the delayed proliferation in the experimental group.

The Device group's average fluorescence differed significantly from the controls' on Day 4, suggesting that PEMF was responsible for a statistically significant inhibition of cell growth ($p < 0.05$). On Day 5, the control group's viability had significantly decreased in comparison to the Device group. This decline in the number of cells in the control group could be due to non-PEMF-related factors like nutrient exhaustion or culture confluence(10).

Line graphs illustrating mean fluorescence (+/- SEM) over time were used to graphically depict trends, making it evident that PEMF-exposed samples had a suppressed growth trajectory.(Figure 4). Individual data points per replicate further support the reproducibility of the results.

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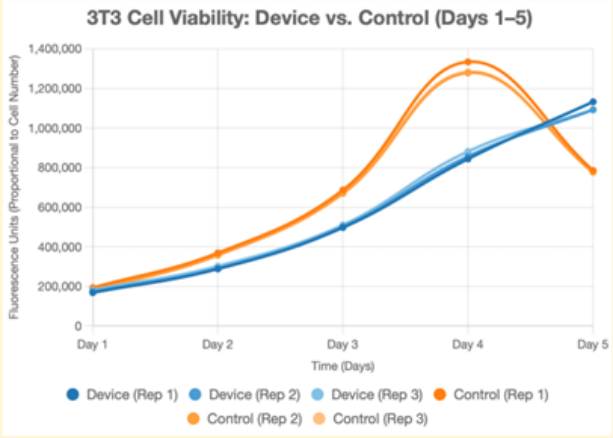

Figure 4- Line graph depicting the gradual changes in cell populations in experimental and control group

5. Discussion

PEMF-induced modulation of cell proliferation is caused by intricate and multifaceted mechanisms. According to the literature, electromagnetic fields may: Modify the production of reactive oxygen species (ROS), which can affect mitogenic and apoptotic pathways; Alter cellular membrane potentials, which can affect signal transduction cascades and ion flux (11) (particularly calcium and potassium channels); Modulate production of reactive oxygen species (ROS), thereby impacting mitogenic and apoptotic pathways(12); Influence gene expression relevant to cell cycle regulation, differentiation, and growth factor responsiveness(13); Affect ATP production and mitochondrial function(14), directly impacting metabolic activity and viability readouts such as the Alamar Blue assay.

Across craniofacial research, the literature underscores that temporomandibular disorders are multifactorial, involving biomechanical, neuromuscular, psychological, and occlusal contributors, with parafunction and trauma prominent among risks(15). On orthopantomograms, population-based imaging reveals significant variation in mandibular condylar morphology, which may have an impact on joint loading and treatment planning.(16). Diverse coronoid shapes are also reported in studies of mandibular processes, mapping the incidence of triangular, hook, and bilobed forms with respect to surgical access, prosthetic design, and coronoidectomy.(17). By providing population-specific baselines, complementary morphometric work supports articulator settings, interarch relationships, and forensic identification by describing Bonwill's triangle dimensions on dry mandibles.(18). Mandibular metrics and asymmetries are further described by additional regional morphometric analyses, which emphasize that anatomical variability has clinical implications for maxillofacial surgery, prosthodontics, orthodontics, and temporomandibular rehabilitation.(19). Together, these studies advocate individualized diagnosis and anatomy-aware, tailored clinical interventions.

Our study demonstrates that exposure to pulsed electromagnetic fields produces a significant and cumulative inhibitory effect on 3T3 fibroblast proliferation in vitro. By the end of the experiment, PEMF-exposed cultures only reach about 66% of control cell numbers, as determined by Alamar Blue fluorescence, indicating that this suppression is noticeable by Day 3 and extremely significant by Day 4. Previous research on different fibroblast populations found that PEMF decreased proliferation and even had pro-differentiative or anti-inflammatory effects.(20). However, when exposed to PEMF, progenitors of bone and cartilage show stimulatory responses.(21).

PEMF's inhibition of fibroblast proliferation may have both medicinal and regenerative uses. Excessive or dysregulated fibroblast proliferation during wound healing can result in fibrosis or hypertrophic scarring(22). PEMF-induced fibroblastic activity modulation may therefore encourage more orderly tissue repair and less scarring; in orthopedic and dental tissue engineering, fibroblast growth control may be helpful for focusing regeneration on particular cell lineages (chondrocytes, osteoblasts) by reducing undesired fibroblastic overgrowth. These findings highlight the need to customize field parameters and exposure schedules to achieve the desired therapeutic outcome and warn against the general application of PEMF across a range of clinical scenarios.

Although the PEMF group grew steadily over the course of the study, the decline in controls points to potential drawbacks of in vitro culture, including nutrient depletion, the buildup of byproducts, culture confluence, and the induction of contact inhibition that results in cellular stress and death. These results emphasize that in order to determine the actual cause of late-stage viability fluctuations, future research must perform simultaneous analyses of direct cell counting, evaluation of

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apoptosis/necrosis, and media replenishment. Compared to osteoblasts or endothelial cells, fibroblasts are typically less sensitive to PEMF-stimulated proliferation, according to systematic reviews and meta-analyses.(23). The idea that electromagnetic field effects are cellularly specific and contextually determined is supported by the observed inhibitory effect in this instance. Additionally, it suggests that abnormal tissue repair, as observed in fibrotic disorders, may be modulable.

The findings add to the debate regarding electromagnetic fields in biomedicine whether they stimulate(24) or suppress(25) cell proliferation. This appears to lie in the exposure attributes and the specific biology of the target cells. The present study reiterates that under certain clinically plausible exposures, PEMF can act as a suppressor of fibroblast activity, which can be leveraged positively in preventing scar overgrowth, or be counterproductive in scenarios demanding high-volume tissue deposition.

Limitations

1. Assay Specificity: Alamar Blue quantifies reductive metabolism rather than direct cell enumeration. Changes in metabolism (not shown as real cell growth or death) could affect the results.
2. Limits on parameters: Only one frequency and intensity were tested: 27.12 MHz and 73 mW/cm². The cellular response to PEMF is very dependent on the parameters.
3. Short Time of Exposure: A 5-day period might miss longer-term adaptive responses or recovery.
4. No Mechanistic Data: There was no analysis of molecular or signaling pathways, such as looking at markers of proliferation, proteins of apoptosis, or gene expression.
5. Cell-Line Specificity: Results obtained from murine 3T3 cells may not be directly applicable to primary human fibroblasts or other cell types.

Future Scope

- Add direct cell counting and cell cycle analysis.
- Evaluate fibroblast responses in three-dimensional or co-culture systems with various cell types to more accurately replicate tissue microenvironments.
- Carry out in vivo studies utilizing animal models of wound healing or tissue regeneration to evaluate clinical applicability.
- Use transcriptomic, proteomic, and metabolomic profiling after PEMF exposure to look into the underlying processes.

Conclusion

The Alamar Blue viability assay shows that exposure to pulsed electromagnetic fields (PEMF) at 27.12 MHz, 73 mW/cm², 12 h/day, and 5 days has a clear suppressive effect on the growth of 3T3 fibroblast cells in vitro. By Day 4, groups exposed to PEMF exhibit markedly diminished cell counts relative to controls, corroborating the hypothesis that electromagnetic stimulation can influence fundamental cellular behavior. Our results are in line with what other studies have found about PEMF's effects on specific cells and in certain situations. They also show how these fields could be used for medical and therapeutic purposes. To find the best exposure parameters, prove the translational potential, and figure out the full range of biological responses to PEMF, more systematic and mechanistic studies are needed.

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