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Anti-inflammatory activity of magnetic fields emitted by graphene devices on cultured human cells

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Abstract

Background Inflammation plays a key role in various diseases such as pancreatitis, cancer, and rheumatoid arthritis. Acute inflammation involves processes like vasodilation, increased vascular permeability, and leukocyte accumulation, which lead to cellular damage due to reactive oxygen species (ROS). Low-frequency electromagnetic fields (ELF-EMFs) have shown potential in reducing oxidative stress and inflammation. This study assesses the effectiveness of a new wearable device containing graphene quantum dots in reducing inflammation and oxidative stress in Jurkat T cells stimulated by lipopolysaccharide (LPS). The device is evaluated for its impact on ROS production and inflammation.

Results The results show that the device significantly lowers ROS levels and reduces the inflammatory response by decreasing pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β. Additionally, the device inhibits LPS-induced iNOS and COX-2 activity and modulates NF-κB signaling, indicating its potential as a therapeutic tool for managing inflammation and oxidative stress.

Conclusion These findings highlight the device's ability to combat inflammation, offering a non-invasive and effective approach for inflammatory diseases.

Keywords Low-frequency electromagnetic fields, Cytokines, Photo-biomodulation, Inflammation, Quantum Dot, Graphene, Jurkat cells

Introduction

Inflammation is an intricate biological process that implies the activation of immune system cells and the release of molecules responsible for triggering of both acute and chronic diseases [1]. Acute inflammation is a rapid, short-term response to harmful stimuli, characterized by blood vessel dilation, increased vascular permeability, and the accumulation of fluids, immune cells, and inflammatory mediators such as cytokines. Soluble factors play a crucial role in attracting immune cells by promoting the expression of adhesion molecules and stimulating cell migration. Inflammatory cells release substantial amounts of reactive oxygen species (ROS),

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which regulate the activity of many genes and enhance the production of inflammatory cytokines [2, 3].

The role of T cells in cell-mediated immunity is complex and involves regulating the secretion of multiple inflammatory molecules such as inflammatory lipid metabolites, nitric oxide (NO), and cytokines. In addition, inflammatory cells release a considerable amount of ROS that regulate several transcriptional genes, further enhancing the expression of proinflammatory cytokines [4, 5].

ROS are produced during oxygen metabolism and consist of molecules such as the superoxide anion, hydrogen peroxide, and hydroxyl radicals. ROS are involved in cell signaling processes but can be harmful as they activate pathological mechanisms such as inflammation, fibrosis and apoptosis [6, 7].

Several studies demonstrate the effectiveness of low-frequency electromagnetic fields in managing inflammation by modulating oxidative stress [8].

Low-frequency electromagnetic fields (ELF-EMF), such as those emitted by the device under study, represent a promising therapeutic tool for managing inflammation and modulating oxidative stress. These fields, characterised by their non-ionizing, athermic, and non-invasive nature, offer a practical and side-effect-free solution for degenerative diseases associated with increased oxidative stress, inflammation, and apoptotic phenomena [9]. ELF-EMF interact with biological systems by modulating ion channels, aquaporins, and redox homeostasis, offering potential therapeutic benefits. EMFs influence transmembrane potentials by altering the flux of ions—such as potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+})—through electro-conformational changes in alpha-helix proteins. This modulation occurs through synchronized oscillations of ion channels in response to applied fields, which enhances membrane permeability and intracellular signaling [10–11].

Additionally, EMFs induce structural oscillations in aquaporins, affecting the flow of water and protons across cell membranes. Research has demonstrated that exposure to EMFs can reduce the production of ROS, which, when overproduced, can damage cellular components by impairing antioxidant mechanisms. Studies have shown that exposure to low-intensity electromagnetic fields can activate systems that regulate oxidative balance, reduce ROS production, and increase the production of the antioxidant enzyme manganese superoxide dismutase (MnSOD) in neuroblastoma cells [12, 13].

In physical therapy, electromagnetic induction is performed using various instruments, such as mats containing solenoids and emitters that concentrate the radial field for local therapies. These instruments require a connection to the electrical network or, in any case, a power supply [9, 14].

The described device, which is wearable and self-powered, has the potential to revolutionize therapies. It consists of a layer mainly containing graphene nanocrystals (quantum dots, QD) on a polyethylene terephthalate support. When ambient light hits the device, it causes electron delocalization, which in turn leads to photon emission by the quantum dots. These semiconductor nanocrystals are characterized by their bandgap energy, which is the energy required to excite an electron and raise it to a higher energy state, creating an electron-hole pair called an "exciton" [14]. When the electron returns to its original state, it releases energy in the form of photons. The size and shape of the nanocrystals affect the properties of the photons, particularly the wavelength: the smaller the nanocrystal, the greater the bandgap energy, and the shorter the emission wavelength. This phenomenon is known as the "Quantum Effect" and allows for the comparison of the excitation and emission properties of quantum dots of various sizes [15–17]. Oxidative stress and inflammation are closely related physiological processes and are often present together in many diseases. In previous works, we demonstrated that graphene quantum dots in wearable devices emit EMFs that enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). These EMF also help restore glutathione metabolism by impacting enzyme levels like glutathione reductase (GR) and glutathione peroxidase (GPX), thereby reducing hydrogen peroxide (H_2O_2)-induced oxidative stress in leukemic cells [10]. In the present study, the aim is to evaluate whether or not the same magnetic fields have an efficacy as an anti-inflammatory response on human cells in culture stimulated by lipopolysaccharide (LPS).

Materials and methods

Device

As reported in our previous work [10], the Device was designed as a circular polyethylene terephthalate (Mylar®) substrate (diameter 2.5 cm) on which an amalgam containing mainly graphene nanocrystals (quantum dots) is layered; due to sunlight, the electro-hole pair that is created determines an emission peak at 800 nm (Fig. 1). The Device displays the excitation wavelength ($\lambda ex = 485$ nm) and the emission wavelength ($\lambda em = 525$ nm), Full Width at Half Maximum (FWHM: 70 nm, 800 nm) and Intensity less than 0.1 mW.

Cell cultures

Jurkat cells, a Human T-cell leukaemia lymphoblastoid cells (DSMZ ACC 282, Braunschweig, Germany) were cultured in RPMI-1640 supplemented with 10% fetal calf serum, L-glutamine (4 mM), penicillin (100 U mL^{-1}), and streptomycin (100 U mL^{-1}). 2×10^5 cells were seeded on six-well tissue culture plates and then cultured in

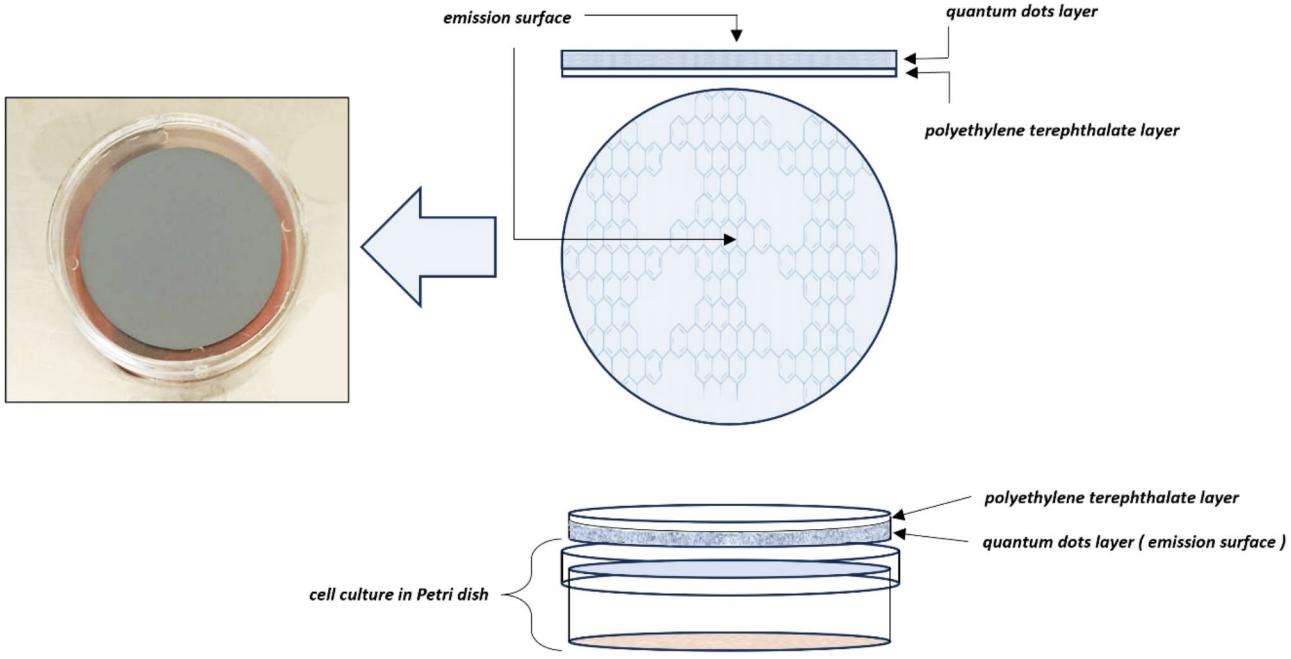

Fig. 1 Device Assembly. It consists of a support made of Polyethylene Terephthalate (Mylar®) that is layered with graphene nanocrystals containing Quantum Dots

medium with and without the application of the Device and/or LPS (10 µg/mL, Sigma).

The LPS was added to the Petri culture dish after the device was positioned 1 cm away. Following a 24-hour incubation period, we evaluated the cellular responses to inflammation by measuring cell viability, the production of pro-inflammatory cytokines, and performing real-time and western blot analysis as detailed below. In an initial trial, we employed a spectrometer (PCE-CRM 40-PCE ITALY s.r.l) to precisely regulate the emission wavelength of the device. The 24-hour exposure duration was determined to effectively counteract inflammation while maintaining constant emission values.

NitroBlue tetrazolium (NBT) assay

The Device's superoxide dismutation potential is assessed using the NBT assay. Cells were seeded onto 96-well culture plates at a density of 5×10^5 cells/mL using a method described previously [18]. Each well was provided with the following components: 100 µL of potassium phosphate buffer (50 mM, pH 7.8), 5 µL of catalase, 25 µL of NBT (5.6×10^{-9} M), 50 µL of xanthine (0.1 mM), and 50 µL of xanthine oxidase (0.1 mM). Following the addition of NBT, the plates were left at room temperature for 1 h to allow the development of the blue color, and the absorbance was then measured at 560 nm.

Western blot analysis

Western blot analysis was carried out as described previously [18], using the following antibodies against, P65 (D14E12; 1:800; Cell Signaling, Danvers, MA, USA), pP65 (93H1; 1:600, Cell Signaling, Danvers, MA, USA), IpKB alpha (NFKBIA) (OTI1D4; 1:400), and β-actin (sc-47778, 1:400; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The membrane was then incubated for two h at room temperature with goat anti-mouse secondary antibody (Sc-2005; 1:2000; Santa Cruz Biotechnology) or polyclonal goat anti-rabbit secondary antibody (Sc-66931; 1:5000; Santa Cruz Biotechnology). The nitrocellulose has been scanned via a computerised densitometric system (Bio-Rad Gel Doc 1000, Milan, Italy). Protein concentrations were normalised to the housekeeping proteins β-actin to adjust for variability in protein loading and expressed as a percentage of vehicle control.

Nitric oxide synthase (NOS) activity

The oxyhemoglobin test was performed to identify the production of nitric oxide from NOS, as outlined in the prior publication [19]. The combination used to measure NOS activity included CaCl2 (1.6 mM), l-arginine (10 µM), calmodulin (11.6 mg/mL), tetrahydrobiopterin (6.5 µM), dihydronicotinamide-adenine dinucleotide phosphate (NADPH, 100 µM), and oxyhemoglobin (3 mM) in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 100 mM) at pH 7.5, in a final volume of 1 mL. iNOS activity was assessed without calcium. The identification of methemoglobin, the result of the reaction between nitric oxide and oxyhemoglobin, was carried out at 576 nm ($e = 12.000\ M^{-1} \cdot cm^{-1}$).

RNA extraction, reverse transcription, and Real-Time PCR

We collected cells in 1 mL of QIAzol lysis reagent (Qiagen, Hilden, Germany) and then isolated total RNA according to the manufacturer's protocol. After that, the concentration of total RNA was assessed using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and one µg of total RNA was transcribed into cDNA using a QuantiTec Revers Transcription Kit, which includes removal of genomic DNA contamination (Qiagen, Hilden, Germany), following the manufacturer's instructions. The cDNA was employed in real-time PCR assays, performed in triplicate using GoTaq qPCR Master Mix (Promega, Madison, WI, USA), as previously explained [20]. The specified conditions included a 2-minute incubation at 95 °C; 40 cycles comprising 30 s at 95 °C; 1 min at 60 °C; and 30 s at 68 °C. Human-specific primer pairs were utilized to assess the expression of the target molecules (Table 1).

Relative expression of each gene was normalised by the 18s gene using the ΔCt method, where $\Delta Ct = Ct_{(COX-2,iNOS,\ TNF-\alpha,IL-1\beta,IL-6,IL-10)} - Ct_{18s}$. Relative fold changes in gene expression were determined by the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct = \Delta Ct_{experimental\ sample} - \Delta Ct_{control\ sample}$.

Cytokines level measurement

The levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, as well as the anti-inflammatory cytokine IL-10, were measured using enzyme-linked immunosorbent assay (ELISA) kits from Proteintech (Proteintech Group, Inc., 5500 Pearl Street, Suite 400, Rosemont, IL, 60018, USA; IL-1β—cat. no. KE00021; IL-6—cat. no. KE00139) and Sigma (IL-10 cat. no. RAB0244). The tests were carried out following the instructions provided by the manufacturers. Each sample underwent analysis in triplicate, and the results were standardized by comparing them with a standard curve.

Table 1 Human-specific primer pairs designed for the evaluation of target molecule expression

Gene	Forward Sequence (5′-3′)	Reverse Sequence (5′-3′)
TNF-α	CCTTCCTGATCGTGGCAG	GCTTGAGGGTTTGCTACAAC
IL-1β	TGAGGATGACTTGTTCTTGAAG	GTGGTGGTCGGAGAATG
IL-6	GAGCTGTGCAGATGATGAGTACAA	GGACTGCAGGAACTCCTTAAA
IL-10	TTTAAGCTGTTTCCATAGGGTGA	TTTATCTTGTCTCTGGGCTTGG
iNOS	CATTGCTGTGCTCCATAGTTTC	CAGGACGTAAGTTCAGCATCTC
COX-2	CGATGCTGTGGAGCTGTAT	TTGAGGCAGTGTTGATGATTTG
18s	CTTTGCCATCACTGCCATTAAG	TCCATCCTTTACATCCTTCTGTC

Measurement of PGE2 release

The cell culture medium was collected at specific time points according to the manufacturer's instructions to quantify PGE2 levels using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI, USA). To elaborate, both control and sample specimens were placed in separate wells and allowed to incubate at room temperature for 15 min. Subsequently, they were incubated overnight at four °C with primary antibody and conjugate. Following the incubation, a washing step was performed, and a substrate solution was added to each well and incubated for 30 min at room temperature. Lastly, a 'stop solution' was added, and the optical density of each well was measured within 30 min using a microplate reader (wavelength 450 nm). The standards employed encompassed a range of 12.5–400 pg/ml for PGE2, with a detection limit of 16.8 pg/ml and a sensitivity of 10.9 pg/ml.

Statistical analysis

The quantitative data was summarized by calculating mean and standard deviation (SD). All results were expressed as means ± standard deviation. Statistical significance was calculated by one-way analysis of variance (ANOVA), and $p < 0.05$ or $p < 0.001$ values were considered statistically significant. The data analysis was performed using GraphPad Prism 8 Software, version 8.4.

Results

Effect of the device on LPS-induced oxidative stress and inflammation

In a previous study, we investigated the impact of the device on Jurkat cells using the MTT assay to determine its cytotoxic effects. The results revealed that a 24-hour treatment with the device did not have any impact on cell viability [10]. Consequently, the device was utilized as a treatment for 24 h for all subsequent experiments. During inflammation, large quantities of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•) are involved in detrimental intracellular processes [21]. To evaluate the device's antioxidant effect against LPS-induced inflammation in Jurkat cells, we assessed ROS production and oxidative stress using the NBT assay, which is commonly used to detect ROS, particularly superoxide anion (O_2^-), associated with cell damage and death [22]. The results of the NBT reduction test are presented in Fig. 2a. LPS stimulation resulted in a significant increase in O_2^- production in Jurkat cells. However, the device inhibited the generation of O_2^- caused by LPS. These findings suggest that the device may help reduce inflammation by lowering ROS levels. To determine if treatment with the device reduces the inflammatory response induced by LPS, pP65 expression levels were analyzed. LPS triggers activation of the NF-κB pathway, where the p65 subunit is crucial as the main

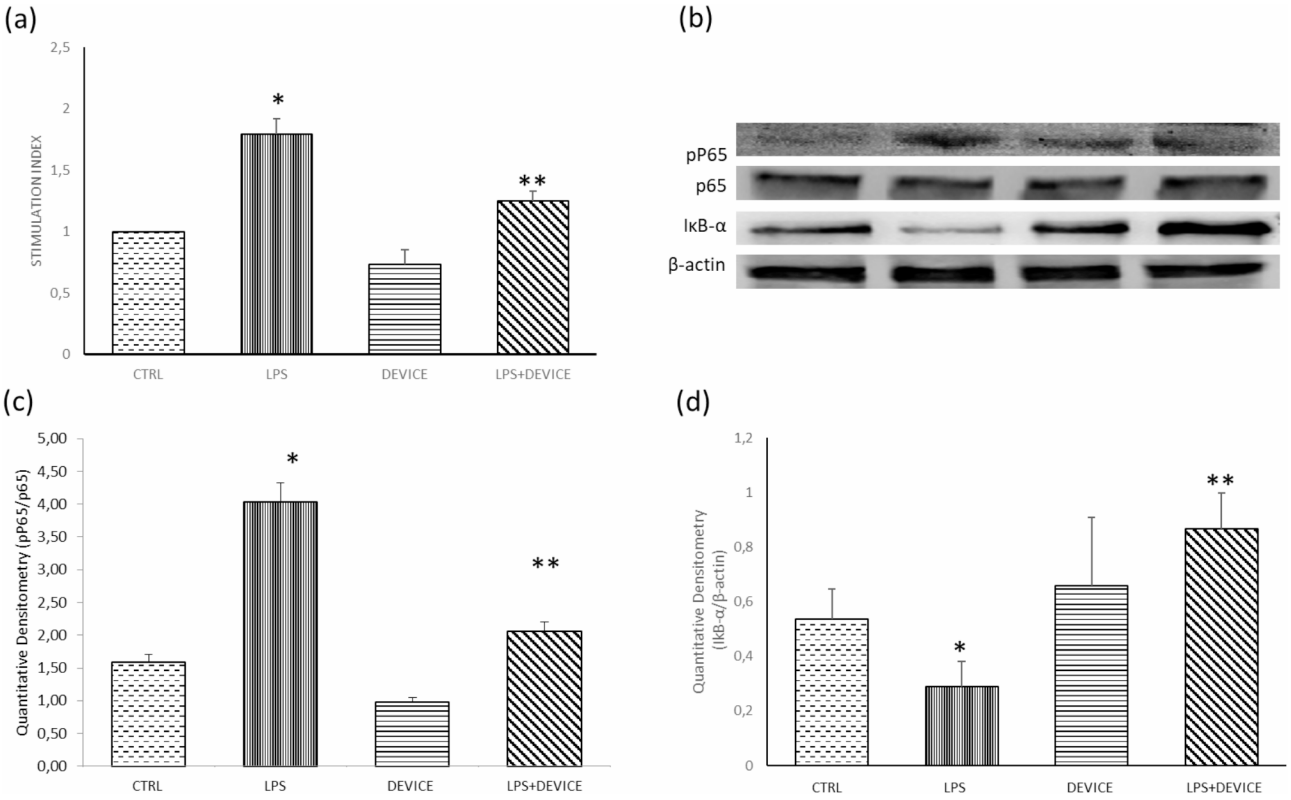

Fig. 2 The impact of the device on superoxide anion and NF-κB signaling in Jurkat cells. (**a**) LPS significantly increases superoxide production in these cells, but the device effectively inhibits this increase. (**b**) Representative Western blotting of p-p65, p65, IkB-α protein expression in cells. (**c**) and (**d**) Graph of the relative density of selected proteins. The LPS leads to the degradation of IκBα, activating the nuclear factor-κB signaling pathway in Jurkat culture. Treatment with the device protects cells from proinflammatory stimuli. Each bar represents the mean ± SD of three groups of cells. *$p < 0.001$ vs. the CTRL. **$p < 0.05$ vs. LPS. CTRL, control; LPS, lipopolysaccharide treated cells

pro-inflammatory mediator, regulating the expression of various NF-κB-dependent genes. These genes encode acute-phase response proteins, inflammatory enzymes such as iNOS and COX-2, and pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β [23]. Figure 2b shows that lipopolysaccharide (LPS) greatly promoted the expression of phosphorylated p65 (pP65), whereas the device treatment significantly abolished pP65 levels. Furthermore, IkBα levels were significantly reduced by LPS stimulation but increased by treatment with the device. Such upregulation of IkBα may further promote its retention with p65. These data support the notion that the device can inhibit the NF-κB signaling pathway by preventing the degradation of IκBα, thereby keeping NF-κB in the cytoplasm and reducing the inflammatory response.

We also analyzed the mRNA expression levels of several key inflammatory factors using qRT-PCR. Treatment with the device downregulated the gene expression of iNOS, COX-2, TNF-α, IL-6, and IL-1β (Fig. 3a). These results were further supported by a notable increase ($P < 0.01$) in the release of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) in LPS-stimulated lymphocytes in comparison to control cells (Fig. 3b). Treatment with the Device significantly decreased the levels of IL-6, TNF-α, and IL-1β in comparison to LPS treatment alone ($P < 0.01$). This validates the potential of the Device to mitigate the inflammatory response by regulating cytokine secretion. In Fig. 3b, it is shown that the presence of LPS significantly increased the production of NO and PGE2 in cells compared to unstimulated control cells. However, when treated with the device, there was a substantial decrease in the production of these inflammatory mediators. Additionally, as reported in Fig. 3a and b, the device treatment did not have a significant impact on the modulation of IL-10 (also known as human cytokine synthesis inhibitory factor, CSIF) expression. Despite the observed reduction in several pro-inflammatory cytokines, the expression and secretion of the anti-inflammatory cytokine IL-10 remained unchanged in Device-treated cells compared to activated cells. This could suggest that the device primarily acts by downregulating pro-inflammatory pathways without directly influencing IL-10-mediated anti-inflammatory mechanisms.

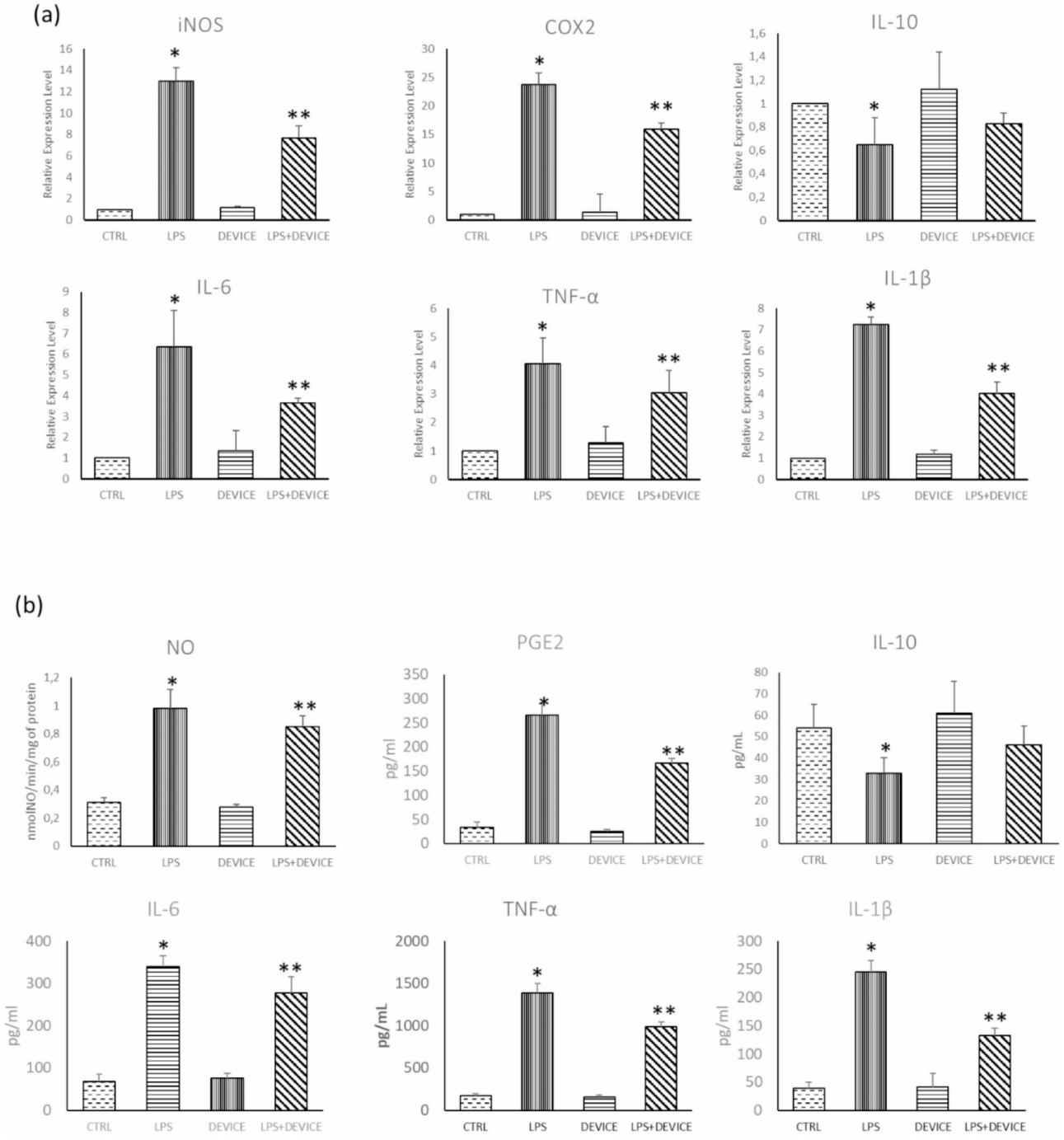

Fig. 3 Impact of Device on NO, PGE2, IL-10, IL-6, TNF-α, and IL-1β Production in Jurkat Cellslipopolysaccharide (LPS)-activated. (**a**) mRNA transcripts for iNOS, COX-2, IL-10, IL-6, TNF-α and IL-1β were identified using by qRT-PCR in Jurkat cells. (**b**) Nitric oxide (NO), prostaglandin E2 (PGE2), interleukin-10 (IL-10), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) levels were measured using an ELISA assay. Results are shown as mean ± SD. *$p < 0.001$ compared to control cells (CTRL); **$p < 0.05$ compared to the LPS-induced group. CTRL, control (unstimulated cells); LPS, endotoxin-treated cells; Device, LPS-treated cells

Discussion

Several studies have demonstrated that weak electromagnetic fields can impact important cellular functions by targeting signal transduction cascades. They can influence cell growth, specific gene transcription, and membrane-mediated signal transduction processes, especially the Ca^{2+} transport system. Several ions are involved in cellular communication and receptor interaction, among which Ca^{2+} plays a central role in modifying the potential of the bilayer constituting cell membranes.

Low-energy electromagnetic fields interact with cellular signaling systems, leading to metabolic responses. This occurs through the modification of signal transduction processes at the membrane level, ultimately causing biochemical perturbations [24, 25].

In vitro studies have investigated signal transduction in response to EMF. The hypothesis that electromagnetic fields interact directly with DNA by activating synthesis through DNA charge transport/flow is increasingly validated.

Since the 1970s, the therapeutic use of EMF has interested the biomedical field, finding the first applications in healing bone fractures and soft tissue injuries. Despite the perception of EMF as potentially dangerous due to adverse effects highlighted in several studies, the progress of research and the increased understanding of the mechanisms have been crucial. This has led to the increasingly defined specific requirements of field energies, confirmed more and more applications, and expanded the range of treatable diseases, making us all part of a dynamic and engaging field [26, 27].

Exposure to low-energy EMF can elicit metabolic responses in the body. This field has the potential to influence the signal transduction processes within cell membranes, leading to both transduction and biochemical amplification of effects.

Interest in the potential of EMFs to influence immune cell responses has been growing. Extensive research, including in vivo, in vitro, and epidemiological studies, has investigated the effects of exposure to extremely low-frequency EMFs on human health, particularly their impact on various biological processes. However, these studies have produced conflicting results, and no clear consensus has been established. It's worth noting that the biological effects of low-frequency EMFs can vary significantly, with both harmful and therapeutic outcomes reported. This offers a glimmer of hope for potential therapeutic benefits, depending on factors such as frequency, amplitude, field strength, exposure duration, and the specific characteristics of the target cells [28].

Free radicals, particularly ROS, play a critical role in promoting inflammation. Conversely, various inflammatory molecules can stimulate ROS production, potentially creating a self-perpetuating cycle of cellular damage, particularly under conditions such as aging or related diseases. This cycle contributes to the deterioration of target organs and cellular damage. Monocytes respond to oxidative stress by upregulating enzymes involved in adaptive survival responses.

The antioxidant capacity of the Device under analysis is primarily derived from its structure, characterized by graphene nanocrystals that can neutralize free radicals, including the superoxide radical. In our previous work, we demonstrated that the Device exhibits antioxidant effects by regulating key enzymes involved in cellular antioxidant defenses [10].

ROS can induce the production of molecules that contribute to inflammation, and conversely, various inflammatory molecules can also promote ROS production. This reciprocal relationship suggests that, under certain conditions like aging or related diseases, a cycle of inflammatory responses may be initiated, leading to cellular damage and deterioration of target organs.

The inflammatory process involves a cascade of reactions triggered by specific receptors that activate a pro-inflammatory transduction pathway, leading to the activation of the Nf-kB complex. This complex is a key regulator of the pro-inflammatory response [29]. Several factors, including bacterial and viral products, cytokines, ultraviolet and ionizing radiation, growth factors, ROS, and oncogenic stress can stimulate NF-κB signaling. LPS stimulation of cells activates the IκB kinase complex (IKK), leading to phosphorylation and ubiquitin-dependent degradation of IκBα, the major inhibitor of NF-κB. When IκBα is degraded, NF-κB translocates to the nucleus and induces transcription of many genes, including those encoding key inflammatory cytokines such as IL-1β, TNF-α and IL-6. This inflammatory state leads to oxidative stress. This in turn increases the production of inducible molecules such as iNOS and COX-2 [23, 30, 31].

The results demonstrated that the Device exhibited an exceptional inhibitory effect on this pathway by limiting NF-κB phosphorylation and its nuclear translocation. In activated cells treated with the Device, the levels of pro-inflammatory factors (TNF-α, IL-6, and IL-1β) were significantly lower compared to cells activated with LPS. Conversely, the level of the anti-inflammatory factor IL-10, which was reduced in inflamed cells, increased significantly following treatment with the Device. These findings suggest that the Device can inhibit the inflammatory process by modulating the secretion of both anti-inflammatory and pro-inflammatory factors.

Conclusion

In this study, the investigated device demonstrated potential in modulating inflammatory processes, presenting a promising non-invasive therapeutic tool without any side effects or needing an external power supply. The device significantly alleviated the inflammatory response induced by LPS and exhibited a strong regulatory effect on the imbalance of inflammatory molecules. It modulates key inflammatory mediators, including iNOS, COX-2, TNF-α, IL-6, and IL-1β, primarily through interference with the NF-κB signaling pathway. These findings suggest that the device may offer a novel approach to managing inflammation and promoting health. The device introduces an innovative approach to treating inflammatory

diseases by leveraging the synergistic effects of an electromagnetic field and the unique properties of graphene. Its non-invasive nature eliminates the need for direct drug administration, potentially reducing the risk of systemic side effects commonly associated with pharmacological treatments.

Limitations and future directions

This study was conducted exclusively using cell lines. Although cell-based models are helpful for preliminary research, the complex interactions present in intact tissues cannot be accurately replicated by them. This limitation restricts our capacity to generalize these results to whole-organism systems. More studies using in vivo models are required to more accurately evaluate the therapeutic potential of the device. In biologically relevant settings, these studies should assess the effects of the device on inflammatory markers, immune cell responses, and tissue recovery over both short- and long-term periods.

More thorough research is required, even though our data points to possible advantages for the treatment of inflammation. To ascertain its wider clinical applicability, future research should examine the performance of the device under a range of pathological conditions, including neuroinflammation, autoimmune disorders, and post-surgical recovery.

In conclusion, even though the device appears to have promise in modifying important mediators of inflammation in vitro, these findings need to be confirmed in more intricate systems before any firm conclusions regarding its potential for treatment can be established.

Abbreviations

ROS	Reactive Oxygen Species
EMF	Electromagnetic Fields
QD	Quantum Dots
LPS	Lipopolysaccharide
O_2^-	Superoxide Anion
H_2O_2	Hydrogen Peroxide
OH•	Hydroxyl Radicals
NBT	NitroBlue Tetrazolium
NOS	Nitric Oxide Synthase
NO	Nitric Oxide
PGE2	Prostaglandin E2
IL-10	Interleukin-10
IL-6	Interleukin-6
TNF-α	Tumor Necrosis Factor-alpha
IL-1β	Interleukin-1 beta

Author contributions

Conceptualization, AG and PDA; methodology, SF; validation, FDC and LS; formal analysis, SB; investigation, SF, FDC and VP; data curation, LS and AG; writing—original draft preparation, AG and SF; writing—review and editing, LS and SB; supervision, PDA. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent Statement for Publication

The authors have given their consent for the publication of this research article.

Competing interests

The authors declare no competing interests.

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References

1. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferrucci L, Gilroy DW, Fasano A, Miller GW, Miller AH, Mantovani A, Weyand CM, Barzilai N, Goronzy JJ, Rando TA, Effros RB, Lucia A, Kleinstreuer N, Slavich GM. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25(12):1822–32. https://doi.org/10.1038/s41591-019-0675-0.
2. Calhelha RC, Haddad H, Ribeiro L, Heleno SA, Carocho M, Barros L. Inflammation: what's there and what's new?? Appl Sci. 2023;13:2312. https://doi.org/10.3390/app13042312.
3. Franceschelli S, Gatta DMP, Ferrone A, Mezza G, Speranza L, Pesce M, Grilli A, Gallorini M, di Giacomo V, Ghinassi B, Fiorito S, Genovese S, Ricciotti E, Felaco M, Patruno A. Anti-Migratory effects of 4'-Geranyloxyferulic acid on LPS-Stimulated U937 and HCT116 cells via MMP-9 Down-Regulation: involvement of ROS/ERK signaling pathway. Antioxidants. 2020;9(6):470. https://doi.org/10.3390/antiox9060470.
4. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic. Biol Med. 2010;49(11):1603–16. https://doi.org/10.1016/j.freeradbiomed.2010.09.006.
5. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239–47. https://doi.org/10.1038/35041687.
6. Rizzoni D, De Ciuceis C, Szczepaniak P, Paradis P, Schiffrin EL, Guzik TJ. Immune system and microvascular remodeling in humans. Hypertension. 2022;79(4):691–705. https://doi.org/10.1161/HYPERTENSIONAHA.121.17955.
7. Muller WA. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. Trends Immunol 24(6) (2003) 327– 34. https://doi.org/10.1016/s1471-4906(03)00117-0
8. Piszczek P, Wójcik-Piotrowicz K, Guzdek P, Gil K, Kaszuba-Zwoińska J. Protein expression changes during phagocytosis influenced by low-frequency electromagnetic field exposure. Int J Biol Macromol. 2022;217:481–91. https://doi.org/10.1016/j.ijbiomac.2022.07.080].
9. Markus I, Ohayon E, Constantini K, Geva-Kleinberger K, Ibrahim R, Ruban A, Gepner Y. The effect of extremely Low-Frequency electromagnetic fields on inflammation and Performance-Related indices in trained athletes: A Double-Blinded crossover study. Int J Mol Sci. 2023;24(17):13463. https://doi.org/10.3390/ijms241713463.
10. Franceschelli S, D'Andrea P, Speranza L, De Cecco F, Paolucci T, Panella V, Grilli A, Benedetti S. Biological effects of magnetic fields emitted by graphene devices, on induced oxidative stress in human cultured cells. Front Bioeng Biotechnol. 2024;12:12:1427411. https://doi.org/10.3389/fbioe.2024.1427411.
11. Bramini M, Alberini G, Colombo E, Chiacchiaretta M, Di Francesco ML, Maya-Vetencourt JF, Maragliano L, Benfenati F, Cesca F. Interfacing Graphene-Based materials with neural cells. Front Syst Neurosci. 2018;12:12. https://doi.org/10.3389/fnsys.2018.00012].
12. Falone S, Marchesi N, Osera C, Fassina L, Comincini S, Amadio M, Pascale A. Pulsed electromagnetic field (PEMF) prevents pro-oxidant effects of H2O2 in SK-N-BE(2) human neuroblastoma cells. Int J Radiat Biol. 2016;92(5):281–6. https://doi.org/10.3109/09553002.2016.1150619.
13. Patruno A, Amerio P, Pesce M, Vianale G, Di Luzio S, Tulli A, et al. Extremely low frequency electromagnetic fields modulate expression of inducible nitric oxide synthase, endothelial nitric oxide synthase and cyclooxygenase-2 in the human keratinocyte cell line HaCat: potential therapeutic effects in

wound healing. Br J Dermatology. 2010;162(2):258–66. https://doi.org/10.1111/j.1365-2133.2009.09527.x.
14. Wagner AM, Knipe JM, Orive G, Peppas NA. Quantum Dots in biomedical applications. Acta Biomater. 2019;94:44–63. https://doi.org/10.1016/j.actbio.2019.05.022].
15. Hartard M, Fenneni MA, Scharla S, Hartard C, Hartard D, Mueller S, Botta Mendez G, Ben Saad H. Electromagnetic induction for treatment of unspecific back pain: A prospective randomized Sham-Controlled clinical trial. J Rehabil Med. 2023;55:jrm00389. https://doi.org/10.2340/jrm.v55.3487.
16. Granada-Ramírez DA, Arias-Cerón JS, Rodriguez-Fragoso P, Vázquez-Hernández F, Luna-Arias JP, Herrera-Perez JL, Mendoza-Álvarez. JG. 16-Quantum dots for biomedical applications. Nanobiomaterials (2018) 411–436. https://doi.org/10.1016/B978-0-08-100716-7.00016-7
17. Babu LT, Paira P. Current application of quantum Dots (QD) in Cancer therapy: A review. P. Mini Rev Med Chem. 2017;17(14):1406–15. https://doi.org/10.2174/1389557517666170315125504.
18. Franceschelli S, Gatta DM, Pesce M, Ferrone A, Patruno A, de Lutiis MA, Grilli A, Felaco M, Croce F, Speranza L. New approach in translational medicine: effects of electrolyzed reduced water (ERW) on NF-κB/iNOS pathway in U937 cell line under altered redox state. Int J Mol Sci. 2016;17(9):1461. https://doi.org/10.3390/ijms17091461.
19. Maccallini C, Patruno A, Lannutti F, Ammazzalorso A, De Filippis B, Fantacuzzi M, Franceschelli S, Giampietro L, Masella S, Felaco M, Re N, Amoroso R. N-Substituted acetamidines and 2-methylimidazole derivatives as selective inhibitors of neuronal nitric oxide synthase. Bioorg Med Chem Lett. 2010;20(22):6495–9. https://doi.org/10.1016/j.bmcl.2010.09.059.
20. Franceschelli S, Gatta DMP, Pesce M, Ferrone A, Quiles JL, Genovese S, Epifano F, Fiorito S, Taddeo VA, Patruno A, Grilli A, Felaco M, Speranza L. Modulation of CAT-2B -Mediated l-Arginine uptake and nitric oxide biosynthesis in HCT116 cell line through biological activity of 4′-Geranyloxyferulic acid extract from Quinoa seeds. Int J Mol Sci. 2019;20:3262. https://doi.org/10.3390/ijms20133262.
21. Franceschelli S, Pesce M, Ferrone A, De Lutiis MA, Patruno A, Grilli A, Felaco M, Speranza L. Astaxanthin treatment confers protection against oxidative stress in U937 cells stimulated with lipopolysaccharide reducing O_2^- production. PLoS ONE. 2014;9(2):e88359. https://doi.org/10.1371/journal.pone.0088359.
22. Elferink J. Measurement of metabolic blast in human neutrophils: a comparison of cytochrome C and NBT reduction. Res Commun Chem Pathol Pharmacol. 1984;43(2):339–42.
23. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, Zeng C, Zhou T, Zhang J. NF-κB in biology and targeted therapy: new insights and translational implications. Sig Transduct Target Ther. 2024;9:53. https://doi.org/10.1038/s41392-024-01757-9.
24. Su J, Song Y, Zhu Z, Huang X, Fan J, Qiao J, Mao F, Mao. Cell–cell communication: new insights and clinical implications. Sig Transduct Target Ther. 2024;9:196. https://doi.org/10.1038/s41392-024-01888-z.
25. Luben RA. Effects of low-energy electromagnetic fields (pulsed and DC) on membrane signal transduction processes in biological systems. Health Phys. 1991;61(1):15–28. https://doi.org/10.1097/00004032-199107000-00002.
26. Blank M, Goodman R. Do electromagnetic fields interact directly with DNA? Bioelectromagnetics. 1997;18(2):111–5. https://doi.org/10.1002/(sici)1521-186x(1997)18:2%3C111::aid-bem3%3E3.0.co;2-5.
27. Huang P, Xu L, Xie Y. Biomedical applications of electromagnetic detection: A brief review. Biosensors (Basel). 11 (7)(2021) 225. https://doi.org/10.3390/bios11070225
28. Patruno A, Costantini E, Ferrone A, Pesce M, Diomede F, Trubiani O, Reale M. Short ELF-EMF exposure targets SIRT1/Nrf2/HO-1 signaling in THP-1 cells. Int J Mol Sci. 2020;21(19):7284. https://doi.org/10.3390/ijms21197284.
29. Checa J, Aran JM. Reactive oxygen species: drivers of physiological and pathological processes. J Inflamm Res. 2020;13:1057–73. https://doi.org/10.2147/JIR.S275595.
30. De Cecco F, Franceschelli S, Panella V, Maggi MA, Bisti S, Bravo Nuevo A, D'Ardes D, Cipollone F, Speranza L. Biological response of treatment with saffron petal extract on Cytokine-Induced oxidative stress and inflammation in the Caco-2/Human leukemia monocytic Co-Culture model. Antioxid (Basel). 2024;13(10):1257. https://doi.org/10.3390/antiox13101257.
31. Guo Q, Jin Y, Chen X, Shen X, Lin M, Zeng C, Zhou T, Zhang J. NF-κB in biology and targeted therapy: new insights and translational implications. Sig Transduct Target Ther. 2024;9:53. https://doi.org/10.1038/s41392-024-01757-9.

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