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**EXPLORING ELECTRIC AND MAGNETIC FIELDS AS PROMISING BRAIN
CANCER TREATMENTS: INVESTIGATING THEIR EFFECTS ON
GLIOBLASTOMA CELLS**

Relatore: Prof.ssa Elisa Cimetta

Laureando/a: Silvia Cesaron 2114150

Correlatore: Ing. Sara Micheli

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Abstract

This thesis investigates the potential of electric and magnetic fields as promising non-invasive therapeutic strategies for brain cancer, with a specific focus on their effects on glioblastoma cells. Glioblastoma is a highly aggressive and invasive brain tumor with poor prognosis, despite advances in standard treatments such as surgery, radiotherapy, and chemotherapy. The urgent need for alternative approaches has led to growing interest in the use of electric and magnetic fields as potential non-invasive cancer treatments to disrupt tumor progression.

Previous studies suggest that these fields may selectively inhibit tumor growth and metastatic spread by interfering with mitotic processes and altering membrane potentials. Building on this foundation, the present study aimed to evaluate the effects of electric and magnetic fields on glioblastoma cells and astrocytes, assessing changes in cell viability, proliferation, morphology, and metabolism.

A systematic methodology was adopted: the setups used to generate the fields were first characterized, followed by numerical simulations to confirm accurate intensity and frequency generation. Cell cultures were then exposed to the fields under controlled conditions, and their responses were assessed using a range of biological assays.

Experimental results demonstrated that exposure to electric fields significantly reduced glioblastoma cell proliferation and viability. Both glioblastoma cells and astrocytes exhibited stress responses, including morphological changes and cytoskeletal disruption. Interestingly, glioblastoma cells showed greater short-term resilience, whereas astrocytes experienced more pronounced reductions in viability. High-intensity magnetic field exposure led to severe stress responses and growth inhibition in both cell types. However, at intermediate intensities, astrocytes exhibited better recovery capacity than glioblastoma cells, suggesting a differential sensitivity to magnetic field-induced stress.

In conclusion, this study provides evidence that electric and magnetic fields can selectively modulate glioblastoma and astrocytes behavior, supporting their potential as non-invasive therapeutic tools. While further investigations are necessary, these findings contribute with valuable insights into the emerging field of electromagnetic-based therapies for glioblastoma.

Riassunto

Questa tesi esamina il potenziale dei campi elettrici e magnetici come strategie terapeutiche non invasive per il trattamento del glioblastoma, con particolare attenzione ai loro effetti sulle cellule tumorali. Il glioblastoma è un tumore cerebrale estremamente aggressivo e altamente invasivo, la cui prognosi rimane critica nonostante i progressi nelle terapie convenzionali, quali chirurgia, radioterapia e chemioterapia. L'urgente necessità di approcci terapeutici alternativi ha portato a un crescente interesse verso l'utilizzo dei campi elettrici e magnetici come possibili trattamenti oncologici non invasivi.

Studi precedenti hanno evidenziato come tali campi possano inibire selettivamente la proliferazione tumorale e la diffusione metastatica, interferendo con i processi mitotici e alterando i potenziali di membrana. Il presente studio si propone di validare gli effetti dei campi elettrici e magnetici sulle cellule di glioblastoma e sugli astrociti, analizzandone l'impatto sulla vitalità cellulare, la proliferazione, la morfologia e il metabolismo. L'approccio sperimentale adottato è stato articolato in più fasi: inizialmente sono stati caratterizzati i sistemi per la generazione dei campi elettrici e magnetici e sono state condotte simulazioni numeriche per garantirne un'applicazione accurata. Successivamente, le colture cellulari scelte (linea cellulare U-87 MG per il modello di glioblastoma e linea cellulare di astrociti umani per simulare il comparto sano) sono state sottoposte a un'esposizione controllata ai campi, e le loro risposte biologiche sono state analizzate mediante diversi saggi sperimentali.

I risultati ottenuti hanno evidenziato che l'esposizione ai campi elettrici ha determinato una significativa riduzione della proliferazione e della vitalità delle cellule di glioblastoma. Entrambe le linee cellulari hanno manifestato risposte di stress, tra cui alterazioni morfologiche e citoscheletriche; tuttavia, le cellule di glioblastoma hanno mostrato una maggiore resilienza nell'immediato post-esposizione, mentre gli astrociti sono risultati più compromessi in termini di vitalità. Diversamente, l'esposizione a campi magnetici di elevata intensità ha indotto una marcata inibizione della crescita e risposte di stress severo in entrambe le popolazioni cellulari, con conseguente distacco dalla superficie di semina e perdita della morfologia originaria. Tuttavia, a intensità di campo intermedie, gli astrociti hanno mostrato una maggiore capacità di recupero rispetto alle

cellule di glioblastoma, suggerendo una differente sensibilità allo stress indotto dai campi magnetici.

In conclusione, i dati sperimentali ottenuti confermano che i campi elettrici e magnetici influenzano selettivamente le cellule di glioblastoma e gli astrociti, supportando il loro potenziale impiego come strategie terapeutiche non invasive. Sebbene siano necessarie ulteriori indagini per chiarire i meccanismi d'azione e ottimizzare i parametri di trattamento, i risultati di questo studio forniscono un contributo significativo alla comprensione delle possibili applicazioni delle terapie basate su campi elettromagnetici nel trattamento del glioblastoma.

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Introduction

Glioblastoma is the most aggressive and common malignant primary brain tumor, characterized by rapid proliferation, high invasiveness, and resistance to conventional therapies. Its survival rate remains extremely poor, with a one-year post-diagnosis survival rate of 65%, which decreases to just 5% after five years, despite significant improvements in neurosurgery, chemotherapy, and radiotherapy. The highly heterogeneous nature of glioblastoma, along with its ability to infiltrate surrounding brain tissue, presents significant challenges for effective treatment. As a result, innovative therapeutic approaches are being explored to improve patient outcomes.

In recent years, the application of electric and magnetic fields has emerged as a promising non-invasive strategy for brain cancer treatment. Electric fields have been shown to interfere with cell division, particularly by disrupting mitotic spindle formation and inhibiting proliferation. This approach has led to the development of tumor-treating fields (TTFields), which have demonstrated efficacy in slowing down glioblastoma progression in clinical settings. Similarly, magnetic fields have been investigated for their potential to modulate cellular behavior, influence ion channel activity, and induce stress responses that affect cell viability and metabolism.

The project described in this thesis was conducted at the BIAMET (Biomedical Applications of Multiscale Engineering Technologies) laboratory, which combines biological and engineering knowledge to study human cancer.

This thesis aims to contribute to this growing field by evaluating the effects of electric and magnetic fields on glioblastoma cells and astrocytes. It is structured into three chapters. The first provides an overview of electric and magnetic fields as innovative, non-invasive therapeutic approaches, with a specific focus on glioblastoma. The second chapter details the methodologies and materials used in the experiments, characterizing the experimental setup and explaining the protocols performed for biological validation. Finally, Chapter three presents and discusses the results obtained from this thesis project.

Chapter 1

State of the art

In this chapter is presented an overview of the state of the art regarding electromagnetic field technology and its use in cancer therapy. In particular the two fields will be characterized in order to determine the appropriate intensities and frequencies required to achieve the desired effect on the cells. Subsequently, the different effects of electric and magnetic field on tumoral and healthy cells will be analyzed.

1.1 Electromagnetic field technology

In physics, the electromagnetic field (EMF) is a field that describes the electromagnetic interaction. It is generated by a combination of the electric and the magnetic fields, both of which vary with time and propagate through space together. These fields are vector quantities, possessing both magnitude and direction. An electric field occurs whenever there is an electric charge, while a magnetic field is generated when electric charges are in motion, such as in an electric current. The strengths of these fields are the highest near their sources and decrease significantly with distance. (Salvatore, 2014)

These fields are intimately linked through Maxwell's equations and together constitute the electromagnetic force, one of the four fundamental forces of the universe.

Electromagnetic fields propagate through space in the form of electromagnetic waves, which are characterized by oscillating electric and magnetic fields. These fields are mutually perpendicular as shown in Fig. 1.1, and to the direction of wave propagation. Electromagnetic waves travel at the speed of light in a vacuum, approximately $3 \cdot 10^8$ m/s, and do not require a medium for transmission, distinguishing them from mechanical waves such as sound waves. The wavelength of an electromagnetic wave is a fundamental property that refers to the spatial distance between two consecutive points that are in phase. It represents, in fact, the distance that the wave covers during one complete oscillation. It is inversely related to the frequency, which means that higher frequencies correspond to shorter wavelengths. These have higher energy and tend to interact more strongly with each material such as X-rays and γ -rays. On the other hand, lower

frequencies are associated with longer wavelengths, which carry less energy and tend to interact more weakly with materials, allowing for deeper penetration - for example, in the case of radio waves.

As previously mentioned, electromagnetic waves vary in both frequency and wavelength, covering a broad spectrum of wave types, including radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays, and γ -rays. These waves differ in terms of energy and have distinct technological applications, each suited to specific uses across various fields.

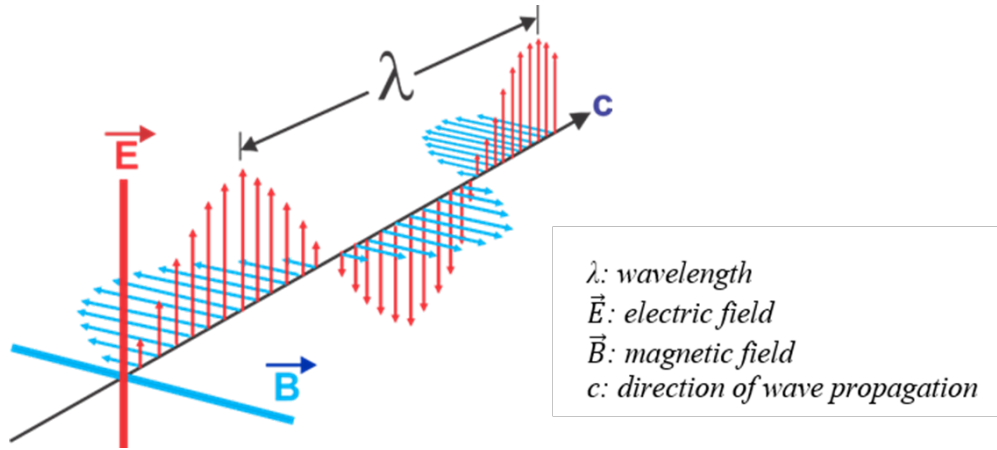


Figure 1.1 Electric ($\vec{E}$) and magnetic field ($\vec{B}$) are mutually perpendicular to each other and to the direction of wave propagation (c).

1.2 Main components of the electromagnetic field

This section presents the main properties of electric and magnetic fields, focusing on the fundamental theory related to both.

1.2.1 Electric field

An electric field is a region of space in which charged particles are subjected to an electrostatic force. The force, acting on an electric charge, is described by the Coulomb's law (1.1), which states that the magnitude of the force between two point charges is directly proportional to the product of their charges (q_1, q_2) and inversely proportional to the square of the distance (r^2) between them:

$$\vec{F} = k_e \frac{|q_1 \times q_2|}{r^2}, \quad (1.1)$$

where k_e is the Coulomb's force constant and it is equal to $8.987 \cdot 10^9 \text{ N} \cdot \text{m}^2 \cdot \text{C}^{-2}$. The direction of the electric field is defined as the direction of the force that the field would exert on a positive test charge placed at a specific point. Its intensity is measured in Volt per meter (V/m).

The spatial distribution of the electric field is represented by the electric field lines. These lines point away from positive charges and towards the negative ones, indicating the path a positive test charge would follow under the influence of the electric field. The density of the field lines corresponds to the strength of the electric field: the closer the lines, the stronger the field.

1.2.2 Capacitor

A capacitor is a device used in electrical circuits to store electric energy. It consists of two conductive plates, known as electrodes, separated by an insulating material called the dielectric. When a voltage is applied across the plates, charges of opposite polarity accumulate on each plate, creating an electric field in the region between them. This allows the capacitor to store energy in the form of an electric field. The capacitor's ability to store energy is quantified by its capacitance, which can be enhanced by using a dielectric material between the two plates.

1.2.3 Magnetic field

A magnetic field is a vector field that describes the magnetic influence of electric currents and magnetic materials. The magnitude of the force exerted on a charged particle by a magnetic field, known as the Lorentz force (F), is proportional to the charge (q), the velocity of the charged particle (v), and the intensity of the applied magnetic field (B), as expressed in the equation (1.2). This equation shows that the force is perpendicular to both the velocity of the particle and the direction of the magnetic field, resulting in circular or helical motion of charged particles in the presence of a magnetic field.

$$\vec{F} = q\vec{v} \times \vec{B} \quad (1.2)$$

A magnetic field is produced by moving electric charges and the intrinsic magnetic moments of elementary particles, which are associated with a fundamental quantum property known as spin. The magnetic field governs the behavior of charged particle in motion and interacts with other magnetic fields. Its intensity is measured in Tesla (T) in the international system (SI).

Static magnetic field are constant fields that do not change in intensity or direction over time, in contrast to low and high frequency alternating fields. Additionally, they have a frequency of 0 Hz.

The magnetic field can be represented through magnetic field lines, which indicate both the direction and the strength of the field. These lines form closed loops, emerging from the north pole of a magnet and re-entering at the south pole. The density of the lines corresponds to the field's strength: the closer the lines, the stronger the field.

1.2.4 Coil

A coil is composed of a conductor wire wound into a spiral, which is used to generate magnetic field. The geometry of the coil, such as the number of turns and the diameter of each loop, is crucial in determining the strength of the magnetic field it produces. When an electric current flows through the coil's conductor, the wire generates a magnetic field around the coil. The intensity of the field is directly proportional to the amount of current passing through the wire. Generally, coils are wound around a magnetic core, which increases the intensity of the magnetic field. The inductance of the coil, which depends on its geometry, determines how much energy the coil can store in the magnetic field.

1.3 Use of the electromagnetic field for cancer treatment

One of the many applications of the electromagnetic fields is in the field of cancer therapy. Tumor treating fields, which involve the use of the electric, and magnetic fields, are emerging as alternative cancer treatments that use a non-invasive approach. These treatments can minimize the effects of chemotherapy, eliminate the probability of infections, reduce treatment costs, etc.

The effects of the electric and magnetic fields can be significantly different, so they will be analyzed separately. The following paragraphs will provide a bibliographical analysis,

reviewing the discoveries made in this field to date and identifying the key areas to focus on for further research.

1.3.1 Characterization of the electric field

Tumor treating field is a non-invasive treatment that applies a low-intensity (1- 4 V/cm), medium-frequency (100-400 kHz) electric field to the tumor (Davies et al., 2013). Due to the dielectric and conductive properties of the biological tissues, the application of an electric field can affect their physiological activities. These frequencies and intensities have primarily been applied to glioblastoma cells.

The medium frequency is used because it is able to interfere with mitosis and other cellular processes. A higher frequency would cause dielectric loss, leading to tissue heating, while a lower frequency would mainly affect the membrane potential of excitable tissue.

1.3.1.1 Exposition time

The exposure time depends on the type of study. Typically, the exposure time for tumor treating treatment (TTtreatment) starts from 1 hour and can extend up to 24 hours. In some studies, where electromagnetic field are also used, exposure times can go up to 72 hours (Berkelmann et al., 2019).

1.3.1.2 Effects on the cell cycle

Many studies have demonstrated that tumor treating field (TTfield) primarily affects proliferating cancer cells by damaging the mitotic processes. The effects on the non-proliferating cells are much lower (Davies et al., 2013). Under dielectric fields, microtubules align with the direction of the electric field, which interferes with normal microtubule polymerization, leading to prolonged mitosis and cell senescence. Additionally, at the end of the mitosis, the daughter cells exhibit an hourglass structure and unevenly distribute the electric field across the dividing cell. This causes chromosomal missegregation during telophase, resulting in abnormal dividing cells with an uneven number of chromosomes, which leads to cell membrane rupture and consequent cell death.

TTfield also increases the foci of DNA double-strand break (DSB) repair and chromatid-type aberrations, disturb the DNA repair, and induce stress during DNA replication.

To explain the cell cycle processes, a study aimed at understanding the relationship between DNA DSBs and single-strand breaks (SSBs) has been reported (Giladi et al., 2017). The researchers combined TTfield with radiation treatment to observe DNA damage and repair in glioma cells. The electric field applied had an intensity of 1.75 V/cm and a frequency of 200 kHz. The cell lines chosen were U-118 MG and LN-18, another type of glioma cells. The application of the TTfield alone for 72h led to over a 50% reduction in the surviving fraction of the U-118 cells, while the LN-18 showed even greater susceptibility, with an $88 \pm 6\%$ reduction in the number of cells. Combining the TTfield effect with the radiation therapy (RT) resulting in a further decrease in the surviving fraction across all radiation doses tested.

It can also be observed that if TTF is applied immediately after the RT, there are greater results than when it is applied after 24 hours. Furthermore, it has been demonstrated that TTfield in U-118 cells delays DNA repair. After 24 hours of treatment, 40% of the DNA damage remained unrepaired, indicating that the TTfield significantly reduces the DNA repair capacity. TTfield also led to a decrease in ATM expression levels (that is one of the earliest activators triggered in response to DNA DSB) and the S1981 phosphorylation pattern (a marker of the DSBs). Additionally, the phosphorylation of histone variant H2AX, another marker of DSBs, was observed. There were no major differences of the γ H2AX foci per cell at 1 hour or 2 hours after the combined treatment compared with RT alone. However, at 24 hours, the number of residual foci was higher in RT + TTfield treated cells than in those treated with either RT or TTfield alone, as shown in Figure 1.2.

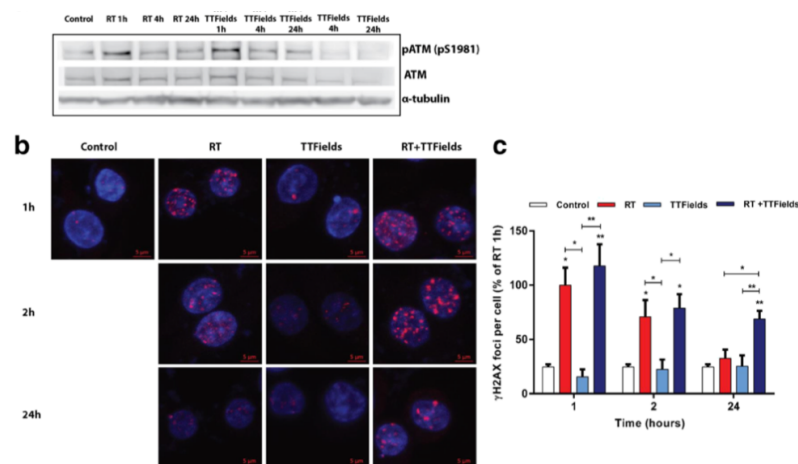


Figure 1.2. TTField Treatment after RT causes the Retention of γ H2AX Foci formation. Adapted from Giladi et al., (2017)

TTfield also disrupts the integrity of the blood brain barrier (BBB), removing obstacles that prevent chemotherapeutic drug delivery into the tumor core, leading to an increased uptake level of the chemotherapeutic drugs.

A study by Chang et al. (2018) found an increase in membrane permeability in glioblastoma cells, indicated by increased bioluminescence after the TTField exposure. The human glioblastoma cell line used was the U87-MG, the electric field intensity was of 1-4 V/cm and the frequency was 200 kHz. As previously stated, TTField primary acts by disrupting cell mitosis by forcing microtubule structures to align along alternating electric field, compromising the functionality of dividing cells and thus inhibiting their proliferation. Moreover, after the TTfield exposure, scanning electron microscopy (SEM) data of the cells that demonstrated an increase in the number of holes in the membrane of the glioblastoma cells, making them more permeable to particles ranging from 4 kDa to 20 kDa. The duration of the TTField exposure ranged from 0.5 to 72 hours. Therefore, TTfield can enhance the antitumor effects of chemotherapy. This effect is reversible after 24 hours.

TTfield can extend survival by inhibiting tumor metastatic spread, seeding, and growth, primarily by preventing angiogenesis and regulating the expression of the epithelial-mesenchymal transition markers (actin, vimentin, and cadherin). It also interferes with tumor migration and downregulates the levels of VEGF, HIF1- α , MMP2 and MMP9 (key factor in tumor growth)

Cancer cells exhibit increased levels of glucose consumption and produce more lactate than normal cells. TTfield inhibits the expression of pyruvate kinase M2, which is associated with elevated glucose uptake, increased lactate production, and reduced oxygen consumption, leading to reduced cell metabolism.

Additionally, TTfield activates calcium channel in the cellular membrane, interfering with electrosignaling that promotes the glioma cells formation (Pei et al., 2020).

1.3.1.3 Devices

To evaluate the safety of TTfields therapy, a medical device has been designed to deliver low intensity and intermediate frequency electric fields to treat recurrent glioblastoma (Davies et al., 2013). The transducer arrays, which incorporate specifically engineered ceramic insulators, a layer of adhesive hydrogel, and a layer of hypoallergenic medical tape, were placed on the patient's shaved head. The patients were treated with a repeated 4-weeks treatment regime, wearing the device 24 hours a day. This treatment showed a survival rate similar to the one obtained with chemotherapy, (Fabian et al., 2019) with the difference that this device improves the quality of life for patients, as shown in the graph in Figure 1.3 (Fabian et al., 2019).

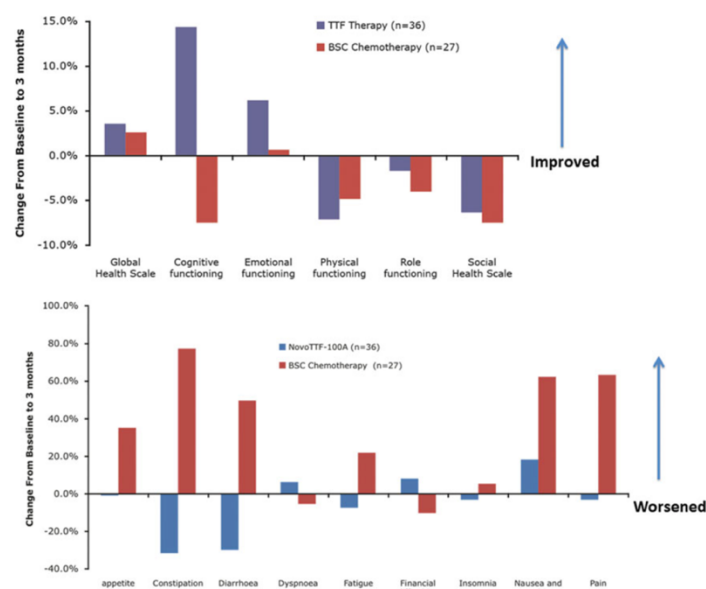


Figure 1.3. Quality of life is higher with the NovoTTF therapy than with chemotherapy. Adapted from Davies et al., 2013.

1.3.2 Characterization of the magnetic field

Epidemiological and laboratory studies suggested that long-term exposure to magnetic field (MF) can increase cancer incidence (Yuan et al., 2018). However, a well-characterized magnetic field is a valid alternative for cancer treatment due to its non-invasive nature and low toxicity. Additionally, it can enhance the efficacy of chemotherapeutic drugs (Lin-Qing Yuan1, 2020).

Magnetic fields can be categorized as either static or dynamic. A dynamic MF, in which a brief current is applied instead of a constant one, induces weak electrical currents in tissues, enhancing the surface of cells and leading to improved blood circulation and oxygenation. It can also generate excessive heat, which could cause thermal damages. However, when used at very low frequencies (ELF, 0-300 Hz), it can generate a strong enough current that is able to stimulate the cells without increasing the temperature. Furthermore, it has been shown to inhibit murine malignant tumor growth by arresting angiogenesis, which is required for tumor growth. Static magnetic fields are preferred to study biological effects due to having fewer parameters (Zhang et al., 2016).

In the following research, magnetic field modulated at different frequencies were used. The intensities typically used are around the 5 mT. In this study, for example, a static magnetic field of 5.1 mT modulated with an ELF-MF (magnetic field at extremely low frequencies) of 50 Hz was applied on human neuroblastoma cells (G401) for 2 hours daily over 3 consecutive days. The results demonstrated an increase in the antitumor efficacy (Yuan et al., 2018). Furthermore, longer daily exposure time always led to higher inhibition rate. In another study (Lin-Qing Yuan1, 2020), the inhibition rate of malignant cell lines was compared to the non-malignant ones, showing that the inhibition rate was higher for the former, as depicted in Figure 1.4. Further study (Sun et al., 2023) investigated the effects of an electromagnetic field combined with a magnetic field (5 mT intensity and 20 Hz frequency) on various cell lines. The fields were applied for 2 hours daily over 3 consecutive days to normal human renal epithelial cells (293T), human liver cancer cells (HepG2), and human non-small-cell lung cancer cells (A549). The results demonstrated a significant reduction in the number of exposed cells compared to the unexposed control group. Interestingly, the reduction in cell numbers was more pronounced in tumoral cells than in non-tumoral cells exposed to the fields.

It is important to remain above 1 mT in order to obtain biological effects, regardless of the frequencies (Yuan et al., 2018).

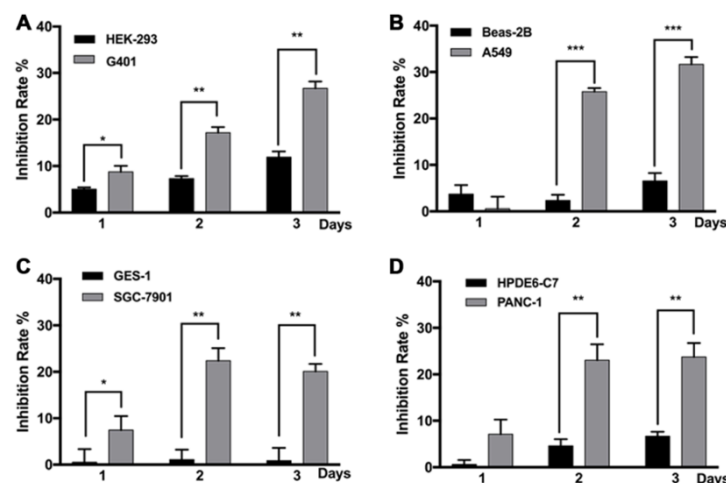


Figure 1.4. MF exposure selectively inhibited the growth of various cancer cell line. Adapted from (Lin-Qing Yuan1, 2020).

1.3.2.1 Exposition time

As mentioned earlier, the experiments reviewed lasted for 3 days, with a daily exposure time of 2 hours. The results demonstrated that the inhibition rate of tumor cells exposed to the magnetic field increased proportionally with the exposure duration. However, it appears that prolonged exposure may lead to the development of resistance mechanisms in the cells, ultimately causing a decline in the inhibition rate curve.

1.3.2.2 Effects on the cell cycle

Several studies have documented that static magnetic fields induce oxidative stress, leading to the damage of cancer cellular membrane ion channels, which triggers apoptosis. The interaction between polar molecules and static MF can generate reactive oxygen species (ROS), which causes oxidative stress and apoptosis in cancer cells. ROS were found after a short-time MF exposure, reaching the peak after 30-60 minutes and maintaining high levels for up to 24 hours after exposure (Sengupta & Balla, 2018).

MFs are also responsible for inducing apoptosis and ferroptosis, a type of programmed cell death (PCD) that is dependent on iron and results from lipid peroxidation.

This process inevitably led to ROS-dependent DNA damage and the subsequent activation of DNA repair pathways. DNA damage was detected using the Alkaline Comet

assay, revealing both double-strand and single-strand breaks, with double-strand breaks being more prominent. DNA damage was further confirmed by the partial incorporation of 5-ethynyl-2'-deoxyuridine (EdU), a marker for DNA synthesis, into the cell nuclei (Figure 1.5B) (Lin-Qing Yuan1, 2020). Beyond direct cytotoxic effects, MFs also influence tumor vasculature. While they can induce localized heating that leads to vascular expansion, they have also been found to significantly decrease vascular endothelial growth factor (VEGF) levels, which is crucial for angiogenesis and tumor progression.

Moreover, this thesis aims to investigate whether magnetic field suppression influences ionic signaling. Studies have shown no significant changes in sodium and potassium ion levels; however, alterations in calcium concentrations have been observed. These variations affect membrane potential, which in turn can disrupt intracellular signaling pathways. Since calcium plays a crucial role in cell survival, its dysregulation may ultimately lead to cell death. Changes in membrane potential further regulate voltage-gated calcium channels (VGCCs) (as shown in Figure 1.5A), which are essential for maintaining calcium homeostasis and mediating cellular responses to external stimuli. The precise mechanisms underlying these effects remain incompletely understood due to the complexity of magnetic influences on biological systems. One proposed explanation involves electromagnetic induction, where MFs generate induced currents and electric fields that alter cell membrane voltage and ion channel activity, particularly affecting Ca^{2+} homeostasis (Zhang et al., 2020). Another hypothesis (Liu et al., 2024) considers the Lorentz force, which acts on moving charged particles, modifying membrane permeability and potentially interfering with mitotic processes by altering microtubule dynamics. Additionally, magnetic fields can influence the orientation of biomolecules with intrinsic magnetic properties, such as DNA and tubulin, further impacting cellular functions. The effects described are summarized in Figure 1.5.

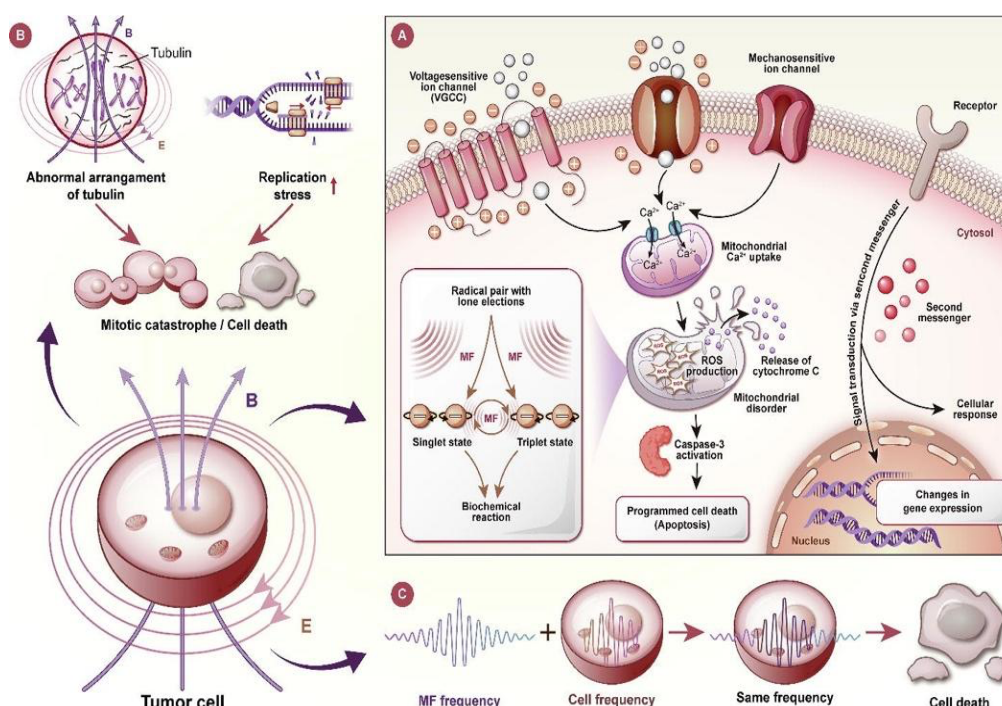


Figure 1.5 (A) LF-MFs can induce ion influx by opening ion channels, such as VGCCs and mechanosensitive channels, and generating Lorentz forces on charged particles. This can trigger apoptosis through calcium-mediated mitochondrial pathways and influence biochemical reactions by altering radical pair states. Additionally, LF-MFs may affect signal transduction via cell receptors. (B) They can also disrupt tubulin and DNA organization, interfering with mitosis and promoting tumor cell death. (C) A resonance effect may enhance biological responses when the frequency of the magnetic field matches that of biochemical reactions. Adapted from (Liu et al., 2024).

1.3.2.3 Effects of conditioned medium after MF exposure

Another study suggested that the different cellular environment could influence the effects of the magnetic field and, consequently, the inhibition rate (Sun et al., 2023). In this study, cells were divided and cultured in two different media before exposure to the magnetic field. The first group, called the “infusion group”, was cultured in a media composed of 500 μ L of fresh medium added to the old one. The second group, the “change group”, was cultured in a completely fresh media, with the old one entirely removed. The “infusion group” had a more complex composition, while the “change group” was cultured in a medium similar to an unused one. After exposure to the magnetic field, results showed that the inhibition rate in the infusion group was significantly higher than in the change group, as observed in Figure 1.5. Tumor cells were more sensitive to the magnetic field in the conditioned medium due to the microenvironment created, which

was modified by autocrine and paracrine signals. The main reason for this is that conditioned medium was more suitable for the growth of cells (in the absence of magnetic field) and facilitated cell-cell communication, which increased contact inhibition under exposure. Therefore, it was demonstrated that the magnetic inhibition rate depends on the stability of the environment.

Additionally, the magnetic field affects the cellular medium itself. In the same study, conditioned medium from cells exposed to the magnetic field was added to unexposed cells (both normal and tumoral). While normal cells showed no inhibition, the tumor cells did. Further experimental verification is needed to clarify the relationship between conditioned media and contact inhibition

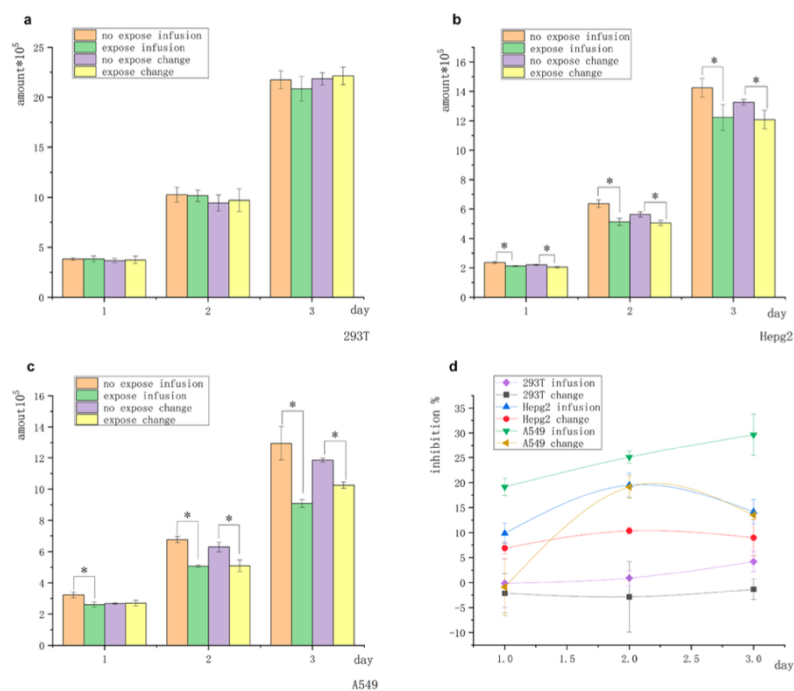


Figure 1.5 Difference in the environment (conditioned medium) before exposure affected the inhibitory effect of the magnetic field on adherent cells. Adapted from (Sun et al., 2023).

1.3.2.4 Spontaneous aggregation and magnetic field inhibition

As previously mentioned, the inhibitory effects of the magnetic field were achieved through cell-to-cell contact and communication. This was demonstrated by studying the effects of cell aggregation using Raji lymphoma cells, where increased cell agglomeration enhanced magnetic field inhibition. These findings suggest that direct cell contact plays

a crucial role in the efficacy of the treatment by impeding tumor cell growth (Sun et al., 2023).

1.3.2.5 pH variations

Limited information is available on this topic; however, the previously cited study showed that the pH of A549 cells increased under magnetic field exposure (Sun et al., 2023). This effect may be attributed to the high sensitivity of this particular cell line to the magnetic field, which could explain why calcium levels were not synchronized with membrane potential.

1.4 Glioblastoma

As reported by Chang et al. (2018), glioblastoma is the most common and most lethal primary brain tumor, accounting for 57% of all gliomas and 48% of all primary malignant tumors of the central nervous system (CNS) (Tan et al., 2020). In the United States, the average yearly incidence of glioblastoma, adjusted for age, is 3.21 cases per 100,000 people, based on registry data from 2011 to 2015. Incidence varies by age and sex, with a median diagnosis age of 65 years and the highest rates observed in individuals aged 75 to 84 years. Globally, glioblastoma incidence is highest in North America, Australia, and Northern and Western Europe.

Its survival rate remains extremely poor, with a one-year post-diagnosis survival rate of 65%, which decreases to just 5% after five years, despite significant improvements in neurosurgery, chemotherapy and radiotherapy (Kim et al., 2016). To date, the main obstacles for the GBM treatment are the blood brain barrier which limits the entrance of the drugs and the tumor heterogeneity which reduces the monotherapy effectiveness and can also lead to drug resistance. These challenges are also compounded by the numerous side effects associated with many therapeutic agents which significantly reduce the patient's quality of life (Liu et al., 2024). Therefore, it is crucial to find alternative therapies to overcome these limitations.

Electric and magnetic exposition has shown great promises as a non-invasive and innovative alternative therapeutic approach for tumors, as explained in §1.3. Specifically for glioblastoma, these therapies have been shown to overcome the previously mentioned obstacles and can be combined with other treatment such as radiotherapy or chemotherapy, to optimize the therapeutic outcomes. In particular, tumor treating fields

treating fields (TTF) consist of low-intensity, alternating electric fields through transducer arrays placed on the scalp to exert an antimitotic effect. When used alongside maintenance therapy with temozolomide, a chemotherapeutic drug, this therapy has been shown to prolong survival in patients with glioblastoma (Tan et al., 2020). A phase 3 clinical trial demonstrated that the combination of TTFs with maintenance temozolomide significantly improved progression-free survival (PFS), increasing it to 6.7 months compared to 4.0 months with temozolomide alone. Moreover, an overall survival (OS) benefit was observed, with a median survival of 20.9 months in the TTF plus temozolomide group, compared to 16.0 months in the temozolomide-only group.

Furthermore, the application of low-frequency magnetic fields (LF-MF) in glioblastoma treatment has shown promising results. Studies have reported that LF-MF can improve the patient's quality of life and reduce the peritumoral edema in the surrounding areas of recurrent glioma (Liu et al., 2024). Additionally, as reported in a study by Baskin et al. (2021), an oscillating magnetic field was applied through a non-contact oncomagnetic device to treat a patient with recurrent glioblastoma, resulting in a 31% reduction in tumor volume by the 31st day. Given these findings, further investigation and analysis of electric and magnetic field-based therapies are crucial, as they have the potential to significantly impact glioblastoma treatment and improve patient outcomes.

1.5 Aim of the thesis

As outlined in this chapter, electric and magnetic fields hold significant promise as a non-invasive approach to cancer treatment. The aim of the thesis is to understand the effects of the two fields on glioblastoma cells and astrocytes. To model tumor and healthy brain tissue, we have selected two specific cell lines: one representing glioblastoma cells and the other representing non-tumor astrocytes, simulating the healthy brain environment. The goal is to demonstrate that the two fields exhibit inhibitory effects only on tumor cells, while leaving the healthy cells unaffected. To achieve this, a series of experiments will be conducted using an appropriate experimental setup capable of providing the desired frequencies and intensities for both the magnetic and electric fields. The effects of the fields will be assessed by evaluating key cellular parameters such as viability, proliferation, morphology, and potential alterations in metabolic activity. These findings

could provide valuable insights into the mechanisms of action of electromagnetic fields and their potential integration into glioblastoma treatment strategies

Chapter 2

Materials and methods

This chapter provides an overview of the characterization of the magnetic and electric field generators. The biological validation is then presented, which includes the illustration of the cell lines chosen in the study and the different protocol adopted.

2.1 Experimental set up

This section describes the characterization of the two experimental systems designed to generate the specific electric and magnetic field. Additionally, Comsol Multiphysics simulations will be presented to verify that the generated fields meet the required parameters.

2.1.1 *Electric field generator*

The electric field is generated using a capacitor connected to a signal generator, which produces a sinusoidal wave with a peak-to-peak voltage of 17 V and a frequency of 200kHz. According to the values reported in §1.3.1, a peak-to-peak voltage of 80 V is required to ensure an electric field intensity of 1-2 V/cm in the petri dishes. To achieve this, the generator is connected to a voltage transformer that increases the voltage to the desired level. The selected capacitor, shown in Fig 2.1, is a parallel-plate type with a capacitance of 12.7 pF. Its plates are made of aluminum, a highly conductive material chosen for its excellent electrical conductivity, corrosion resistance and low density.

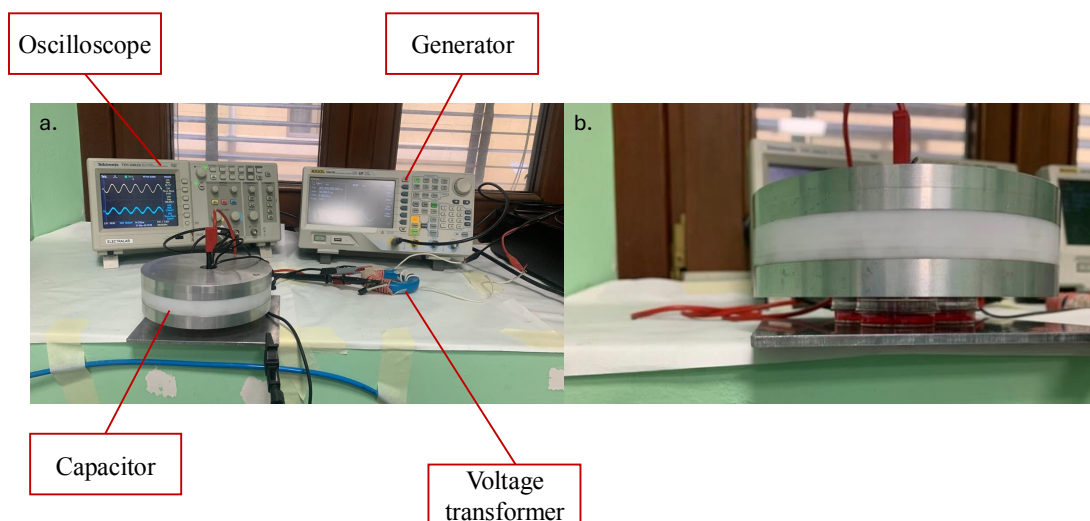


Figure 2.1 Electric field generator. (a) Experimental setup of the capacitor used in the project; (b) Zoomed view of the capacitor where the petri dishes containing the different cell lines are placed between the two plates.

The petri dishes containing the cells are positioned inside the capacitor, between the two plates. The electric field to which the cells are exposed is directed from the top plate to the bottom one, striking the petri dishes perpendicularly. The distribution of the electric field lines is shown in Fig. 2.2.

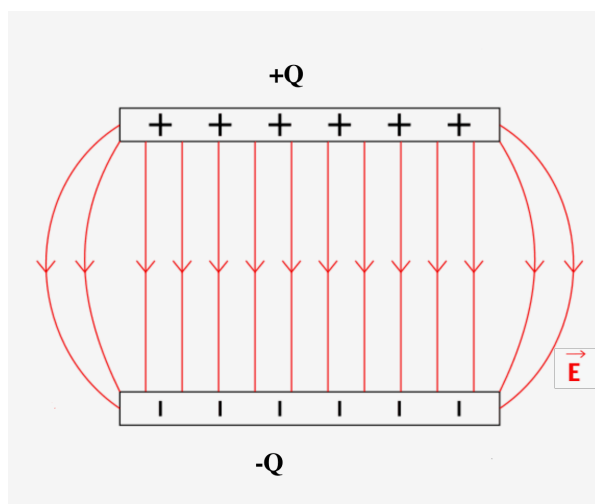


Figure 2.2 Representation of the distribution of the electric field inside a capacitor. The electric field lines extend from the positively charged plate to the negatively charged one. Since the capacitor is used in an alternating current (AC) circuit, the direction of the field lines alternates between top-to-bottom and bottom-to-top, following the frequency of the AC signal. This occurs because the plates of the capacitor periodically change polarity as the AC voltage oscillates, leading to a continuous reversal of the electric field direction.

2.1.1.1 Electric field exposure: working parameter selection

After the bibliographical research reported in §1.3.1, the working parameters chosen for the electric field exposure of the different cell lines are:

- Intensity: 1 V/cm
- Frequency: 200 kHz
- Exposure time: 24 hours

These values have shown promising results in several studies, demonstrating significant inhibition of tumor cell growth. The choice of 200 kHz is based on its effectiveness in disrupting mitotic spindle formation, leading to reduced proliferation and increased apoptosis in glioblastoma cells, as reported by Kim et al. (2016) and subsequent studies. The intensity of 1 V/cm has been selected to ensure sufficient field penetration while maintaining cellular viability during exposure, in line with the findings of Davies et al., (2013) and Cai et al. (2024).

Moreover, these parameters align with clinically approved treatments, such as Tumor Treating Fields (TTFields), which have been shown to enhance patient survival in glioblastoma therapy (Davies et al., 2013). The combination of these frequency and intensity values provides an optimal balance between therapeutic efficacy and minimal adverse effects on non-tumoral cells. The exposure time of 24 hours is appropriate for observing the described phenomena. (Giladi et al., 2017).

2.1.2 Magnetic field generator

The magnetic field generator consists of a coil connected to an amplifier, which is in turn connected to a signal generator, as shown in Fig. 2.3. The signal generator produces a sinusoidal wave with a frequency of 130 Hz and a peak-to-peak voltage of 13 V. To achieve the desired parameters specified in the literature (§1.3.2), the signal is amplified before being supplied to the coil, ensuring a current of 2.1 A.

The coil assembly comprises two concentric cylinders made of polylactic acid (PLA), with a layer of fiberglass wool positioned between them. The outer cylinder is wrapped with 250 turns of copper wire, a configuration designed to maintain a current of 2.1 A. The fiberglass wool layer serves as thermal insulation, preventing heat transfer from the

wire to the petri dishes placed inside the inner cylinder. This setup allows for the generation of a magnetic field with an intensity of 5–7 mT, depending on the position of the petri and the actual density of the copper wires.

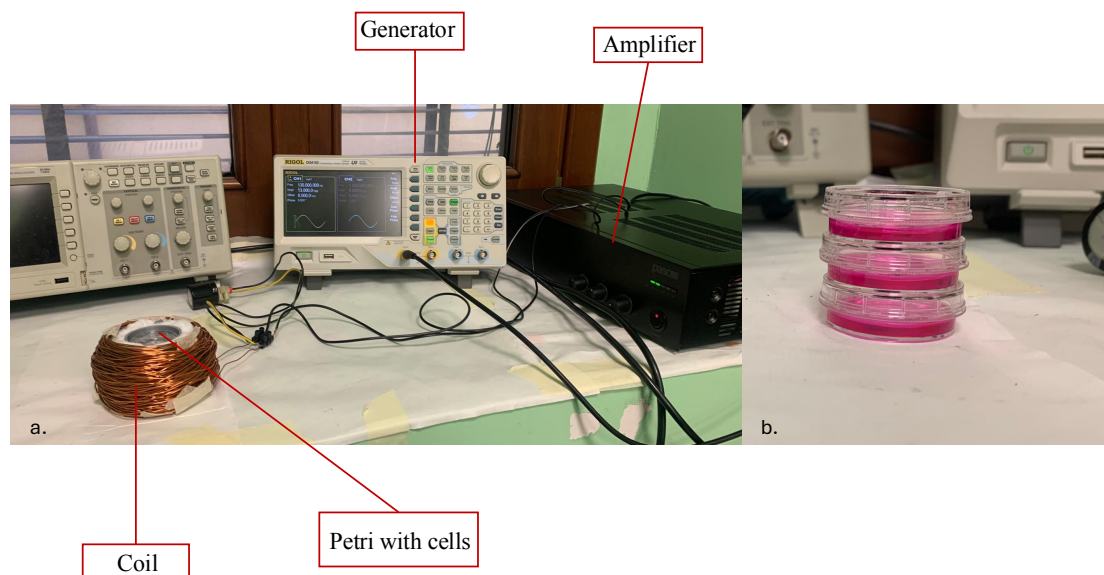


Figure 2.3 Experimental set up to generate the magnetic field. **a.** Experimental set up of the magnetic field generator, showing the amplifier, the signal generator, the coil and the petri with cells inside it. **b.** Photo showing the bottom, middle, and top petri dishes as they were positioned inside the coil during the experiment.

2.1.2.1 Magnetic field exposure: working parameter selection

As reported in paragraph §1.3.2, after a bibliographical analysis, the working parameters chosen for the magnetic field exposure of glioblastoma tumor cells are as follows:

- Intensity: 5-7 mT (varies depending on the position of the petri dishes inside the coil. The petri dish at the top receives the higher intensity, 7 mT, while the middle receives an intensity of 6 mT and finally the bottom dishes receive an intensity of 5 mT).
- Frequency: 130 Hz.
- Exposure time: 3 hours per day for three consecutive days.

These values have shown promising results in vitro. A frequency of 130 Hz falls within the range of extremely low-frequency magnetic fields (ELF-MFs), which can alter ion concentration across the cellular membrane, increase oxidative stress, and activate apoptosis. An intensity of 5-7 mT induces reactive oxygen species (ROS) production,

which interferes with cell viability and promotes DNA damage. Moreover, the exposure schedule of 3 hours per day for three consecutive days was selected to allow for cumulative effects while preventing excessive cellular adaptation, in line with protocols commonly used in the literature (Liu et al., 2024).

2.1.3 Preparation of the PDMS support

Given the numerous tests to be performed, it is useful to design a system that increases the number of replicates within the same experiment, reduces the quantity of reagents required, and allows for more efficient assay execution. A PDMS support was created for this purpose, enabling the seeding of three separate coverslips inside a petri dish. This setup allows for either the culture of two different cell types—astrocytes and U87 cells—as well as their co-culture, or the generation of three replicates of the same cell type within a single petri dish. The chosen material, polydimethylsiloxane (PDMS), is a polymer belonging to the silicone class. It is widely used in biological applications due to its unique combination of biocompatibility, optical transparency, flexibility, and ease of fabrication. Its non-toxic nature makes it suitable for cell culture, while its optical transparency allows for fluorescence testing and makes it visible under a microscope. PDMS is prepared by mixing a curing agent with the silicon base in an 11:1 weight ratio. The support was designed using AutoCAD® to precisely define its dimensions, as shown in Figure 2.4(a). The geometry was designed to fit inside a 35 mm petri dish and consists of three cavities, each accommodating a 12 mm glass coverslip. Once designed, the mold was printed using a Prusa® 3D printer with acrylonitrile butadiene styrene (ABS), as shown in Figure 2.4(b). The PDMS solution was then casted into the mold and cured in an oven at 70 °C for 1 hour until complete crosslinking.

After curing, the PDMS was removed from the mold. A 12 mm biopsy punch was used to create openings in each cavity, providing space for the coverslips, as shown in Figure 2.4(c). The PDMS support was then placed inside a 35 mm petri dish and secured by applying a thin layer of PDMS to its edges, which was subsequently cured again to ensure adhesion.

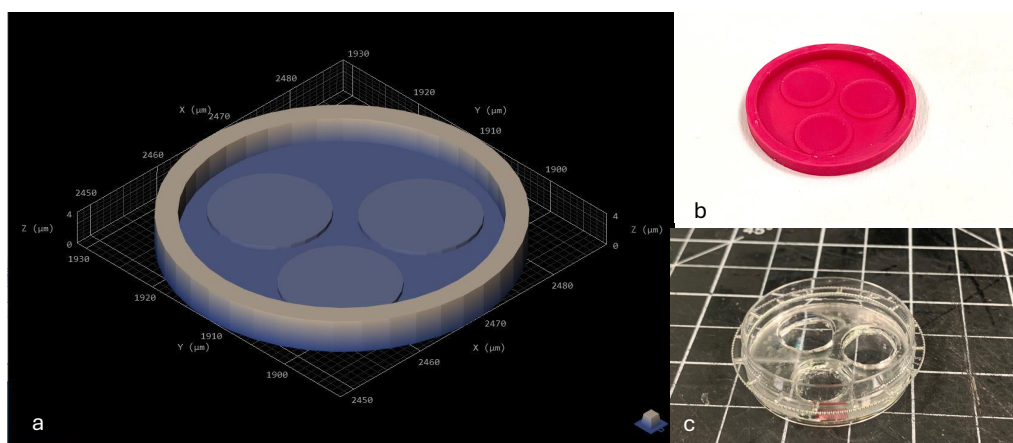


Figure 2.4 PDMS mold realized for the project. (a) 3D AUTOCAD® geometry design of the PDMS support showing three cavities for coverslip placement inside a 35 mm petri dish; (b) 3D ABS printed mold used for PDMS casting; (c) Final PDMS support with openings for coverslips, secured inside a petri dish.

2.1.4 Numerical simulations with COMSOL Multiphysics®

A series of COMSOL Multiphysics® simulations were conducted to optimize the experimental setup and precisely analyze the distribution of both fields within the system. These simulations provide insights into the field distribution inside the petri dish and at the cellular level, as well as evaluate potential unintended heating effects.

2.1.4.1 COMSOL Multiphysics®

The software Comsol Multiphysics® is a simulation software based on the finite element method (FEM) used for modeling and analyzing complex physical systems. It enables the simulation of multiple interacting physical phenomena, such as electromagnetics, fluid dynamics, structural mechanics, and heat transfer, making it suitable for multidisciplinary research and engineering applications.

In this study, COMSOL's AC/DC Module is utilized to simulate the electric and magnetic fields generated by the experimental setups. Specifically, the electrostatics physics interface is used to analyze the electric field, while the magnetic fields interface is employed to simulate the magnetic field. These simulations help verify whether the setups produce the expected field distributions with the desired intensity and frequency, ensuring accurate experimental conditions. To achieve this, the software solves Maxwell's

equations, as shown in equation (2.1), providing a detailed representation of the field distributions.

$$\vec{\nabla} \cdot \vec{\mathbf{E}} = \frac{\rho}{\epsilon_0} \text{ Gauss's law for the electric field (a)} \quad (2.1)$$

$$\vec{\nabla} \cdot \vec{\mathbf{B}} = 0 \text{ Gauss's law for the magnetic field (b)}$$

$$\vec{\nabla} \times \vec{\mathbf{E}} = -\frac{\partial \vec{\mathbf{B}}}{\partial t} \text{ Faraday's law (c)}$$

$$\vec{\nabla} \times \vec{\mathbf{B}} = \mu_0 \vec{\mathbf{j}} + \epsilon_0 \mu_0 \frac{\partial \vec{\mathbf{E}}}{\partial t} \text{ Ampere-Maxwell's law (d)}$$

Maxwell's equations are four differential equations that, along with the Lorentz force law, describe the principles of electromagnetism.

- **Gauss's law for the electric field (a)** states that electric charges generate an electric field. The term ρ represents the charge density, and ϵ_0 is the vacuum permittivity constant. The divergence of the electric field ($\vec{\nabla} \cdot \vec{\mathbf{E}}$) is proportional to the charge density, meaning electric charges act as sources of the electric field.
- **Gauss's law for the magnetic field (b)** states that no magnetic monopoles exist, implying that the total magnetic flux through a closed surface must be zero.
- **Faraday's law (c)** describes how a time-varying magnetic field induces an electric field. The curl of the electric field $\vec{\nabla} \times \vec{\mathbf{E}}$ is proportional to the rate of change of the magnetic field over time $\frac{\partial \vec{\mathbf{B}}}{\partial t}$. This principle underlies electromagnetic induction.
- **Ampere-Maxwell's law (d)** explains how both electric currents ($\vec{\mathbf{j}}$) and time-varying electric fields generate a magnetic field. The first term ($\mu_0 \vec{\mathbf{j}}$) represents the magnetic field produced by an electric current, while the second term ($\epsilon_0 \mu_0 \frac{\partial \vec{\mathbf{E}}}{\partial t}$) describes how a changing electric field also contributes to the generation of a magnetic field. The constant μ_0 represents the vacuum permeability.

These four equations are fundamental to describing electromagnetic waves, the propagation, the behavior of electric and magnetic fields, and their interactions with charged particles and currents.

2.1.4.2 Numerical simulation for the electric field setup using COMSOL Multiphysics®

Before conducting the analysis with Comsol, it is essential to select the appropriate physics and define the parameters to accurately study the phenomenon of interest. In this case, a 2D analysis is performed, as the assumption of axial symmetry allows for simplification. Since the system exhibits symmetry along the y axis, the problem can be reduced to a 2D model instead of a 3D one, significantly simplifying the simulation. Additionally, a stationary study is selected, indicating that the variables do not change over time.

The chosen physics interface is electrostatics, as the objective is to analyze the intensity of the electric field generated by a capacitor on petri dishes containing cell and medium. The experimental setup, shown in Figure 2.5 is designed in Comsol to accurately represent the geometry and conditions of the system.

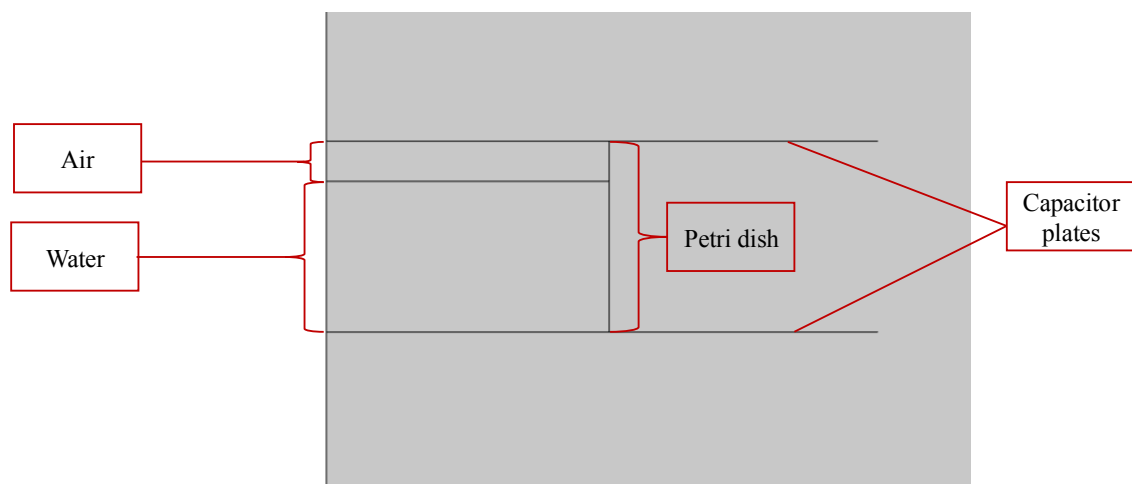


Figure 2.5 Representation of the 2D simulation geometry used in Comsol Multiphysics, showing the petri dish containing e air and water

The materials set in the software and used in the simulation are listed in Table 2.1. The key parameter required is the relative permittivity, which quantifies a material's ability to store electrical energy in an electric field. It is a dimensionless quantity that describes how much a material reduces internal electric field compared to a vacuum. A higher relative permittivity indicates greater polarization of the material, leading to a reduced

electric field inside. This affects both the propagation of electromagnetic waves and the capacitance of electrical components, as explained by the Gauss law's (Equation 2.1a). In this simulation, the cell culture medium is approximated as water due to its similar dielectric properties.

Table 2.1 Value of relative permittivity for the material used in the Comsol simulation.

Relative permittivity	Value	Unit of measure
Water	81	-
Air	1	-
Aluminum	1	-

Due to the high permittivity of water and the lower permittivity of air, it is crucial to completely fill the petri dish to ensure that the electric field is primarily present in the culture media rather than in the surrounding air.

The final step before running the simulation is selecting the mesh. The mesh determines how the model's geometry is discretized for numerical simulations. It must be fine enough to ensure accuracy but not excessively dense, as an excessive number of nodes would increase the computational load. To guarantee reliable results, the mesh should be mesh-independent, meaning that once a proper mesh quality is achieved, further refinement (such as or changing the size or distribution of elements) will not significantly affect the simulation results.

2.1.4.3 Numerical simulation for the magnetic field setup using COMSOL Multiphysics®

As illustrated for the electric field, the magnetic field simulation in Comsol requires careful selection of the appropriate physics and parameters setting to accurately study the phenomenon in question. A 2D model will be employed for this analysis, assuming axial symmetry. Additionally, a stationary study will be selected. The chosen physics interface is *Magnetic Fields*, and the objective is to investigate the intensity of the magnetic field generated by the setup explained in § 2.1.2, which involves petri dishes containing cells and culture medium.

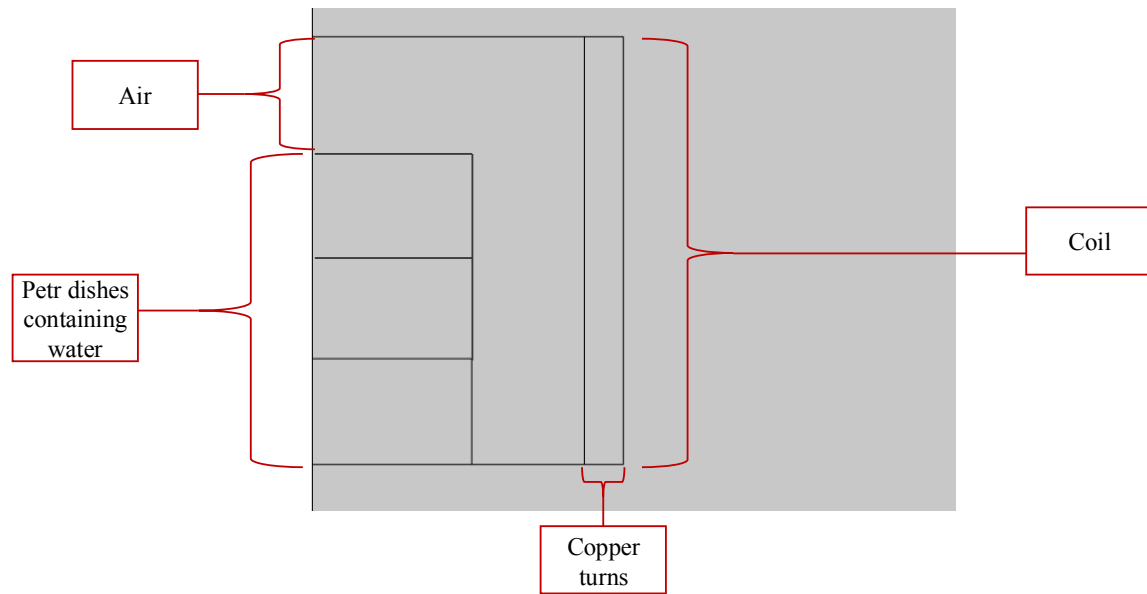


Figure 2.6 2D geometry used in Comsol simulation. The representation of the geometry shows three petri dishes stacked one above the other, each containing water, and a coil composed of 250 copper turns.

After properly designing the geometry within the software (Figure 2.6), material characterization is required. In this simulation, the petri dish is designed to be filled only with water, as the effect of the air inside is negligible.

The parameter required to run the simulation in this case is the relative magnetic permeability. The relative magnetic permeability is a dimensionless quantity that describes how a material responds to a magnetic field in comparison to the permeability of free space (μ_0). The formula is provided in Equation 2.2.

$$B = \mu_r \mu_0 H \quad (2.2)$$

B = Magnetic induction [T]

H = intensity of the magnetic field [A/m] μ_0 = magnetic permeability $4\pi \cdot 10^{-7}$

[H/m] μ_r = relative magnetic permeability

If $\mu_r > 1$, the material is paramagnetic, while if $\mu_r < 1$, the material is diamagnetic. A material is defined paramagnetic if it is weakly attracted to an external magnetic field due to the alignment of unpaired electrons with the field, whereas it is defined diamagnetic if it generates a weak opposing magnetic field when exposed to an external magnetic field. In this simulation, the culture medium is also assumed to be water.

Table 2.2 List of the value of relative permeability for the material used in the Comsol simulation.

Relative permeability	Value	Unit of measure
Water	0.9	-
Air	1.0	-
Copper	0.9	-

The final step involves selecting the mesh. For this simulation, triangular elements are chosen.

2.1.4.4 Numerical simulation for the electric field setup with the PDMS support inside the petri dish using COMSOL Multiphysics®

To increase the number of tests per experiment, a PDMS support has been designed, as explained in § 2.1.3. To assess whether this affects the field parameters reaching the cells inside the petri dish, a Comsol Multiphysics simulation is performed. As described in §2.1.2.2, the same module and physics - namely electrostatics-will be used. In this case, the simulation will be conducted in 3D to gain a deeper understanding of any alterations caused by the PDMS support. The study will remain stationary.

The materials set will be the same as previously used, with the addition of the permittivity of PDMS, as reported in Table 2.3.

Table 2.3 List of the values of relative permittivity used in the Comsol simulation

Relative permittivity	Value	Unit of measure
Water	81	-
Air	1.0	-
Aluminum	1.0	-
PDMS	2.5	-

It can be observed that the permittivity of PDMS (≈ 2.5) is slightly higher than that of air (≈ 1) but significantly lower than that of water (≈ 81). Compared to the previous case, which include only air and water, the introduction of PDMS will likely cause some redistribution of the electric field within it. However, since air has the lowest permittivity,

the field should still predominantly remain there. This will be further explored through simulation.

The geometry used in §2.1.2.2 has been updated to include the PDMS support, which was imported from AutoCAD software.

In the following figure (Figure 2.7), the PDMS geometry is represented in Comsol software, along with the characteristics of the material from which it is made.

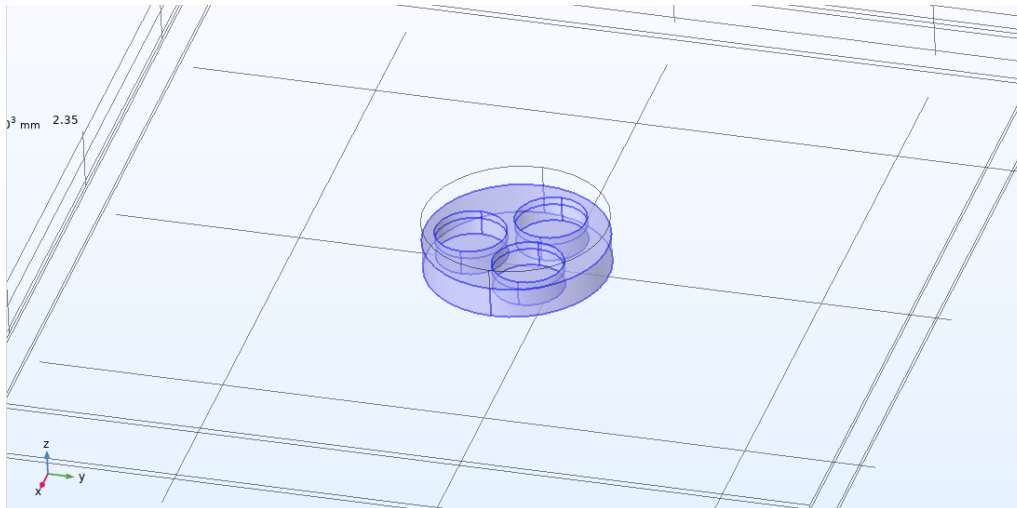


Figure 2.7 Representation of the 3D PDMS support imported in the COMSOL simulation.

The final step consists of selecting the mesh. In this case, since the simulation is in a 3D space, the mesh-independent method chosen is the free tetrahedral mesh.

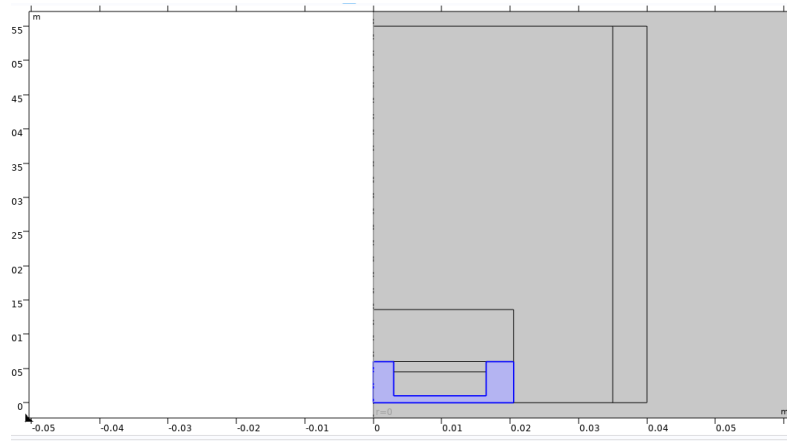
2.1.4.5 Numerical simulation for the magnetic field setup with the PDMS support inside the petri dish using COMSOL Multiphysics®

The PDMS support has been used also in the experiments involving the magnetic field. The goal is to verify that this support does not alter the field parameters. In this case, a 2D analysis will be performed under the assumption of axial symmetry. The stationary study is selected. The chosen physics is the same as in §2.1.2.3, namely magnetic fields. The PDMS support was imported from AutoCAD. Table 2.4 reports the values of relative magnetic permeability used in the simulation.

Table 2.4 List of the value of relative permeability for the material used in the Comsol simulation

Relative magnetic permeability	Value	Unit of measure
Water	0.9	-
Air	1.0	-
PDMS	1.0	-

PDMS has a magnetic permeability close to the one of air, meaning its effect on the magnetic field is expected to be minimal. The geometry from §2.1.2.3 has been updated to include the PDMS support, imported from AutoCAD. Figure 2.8 illustrates the updated design in Comsol, incorporating the PDMS support and also including its material characterization.

**Figure 2.8** Representation of the 2D PDMS support import from AutoCAD in Comsol software inside the petri dish.

The final step consists of selecting the mesh. A mesh independence study was conducted to ensure that further mesh refinement does not significantly affect the results. A free triangular mesh was chosen for this 2D simulation.

2.2 Biological validation

This section explains the protocols for cell counting, splitting, and fixing. Biological validation is necessary to verify cellular adhesion and proliferation in the petri dishes.

2.2.1 Cell lines

In this study, two cell lines were used: U-87 MG, a glioblastoma-derived tumoral cell line, and astrocytes, a specific type of cell in the nervous system.

- **U-87 MG:** This cell line was derived from a male patient diagnosed with glioblastoma and was provided by ATCC. It was maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Waltham, MA, USA), supplemented with 1% v/v Penicillin-Streptomycin (P/S, Sial) and 10% v/v Fetal Bovine Serum (FBS, ThermoFisher). These cells grow in adhesion and were cultured at 37 °C in an atmosphere of 5% CO₂ and 98% humidity. This specific cell line is widely used in brain cancer research.
- **Human Astrocyte (Human immortalized astrocyte):** Star shaped glial cells that constitute the main component of the mammalian central nervous system (CNS) and are involved in various functions, including neuronal support, synaptic regulation and maintenance of the blood-brain barrier. This cell line, provided by Innoprot, was obtained by immortalizing primary human cortical astrocytes with the lentivirus SV40 Large T antigen, allowing them to proliferate indefinitely. The transduced cells can be maintained over 30 passages, whereas primary cells undergo senescence after the fourth passage. These cells grow in adhesion and require prior flask coating with a Poly-L-Lysine (PLL, Innoprot) solution at concentration of 2 µg/ cm². They were maintained in Astrocyte Medium Kit (P60101, Innoprot), a complete medium containing 500 mL of astrocyte basal medium, 10 mL di FBS, 5 mL of astrocyte growth supplement, and 5 mL P/S solution. Additionally, they were cultured at 37 °C in an atmosphere of 5% CO₂ and 98% humidity.

2.2.2 Cellular culturing and splitting protocol

Cells are cultured in 75 cm² flasks, immersed in culture medium, and maintained under controlled conditions to ensure their continuous proliferation. The culturing environment is carefully regulated, maintaining a temperature of 37°C in an atmosphere of 5% CO₂ and 98% humidity. To prevent excessive confluence and maintain a healthy population, cells undergo periodic splitting, a subculturing process aimed at avoiding overcrowding and promoting further growth. Confluence is monitored to prevent cells from occupying

100% of the flask's surface area, as this would induce contact inhibition, eventually leading to growth arrest and cell death. To maintain optimal growth conditions, once it reaches a confluence of 70%-80% the splitting protocol is performed. This subculturing process involves the periodic detachment and reseeding of cells into fresh flasks allowing further growth.

During the splitting procedure, cells can be precisely seeded to obtain the desired number for specific experiments. In this study, as explained in the following paragraph §2.2.4, the cells are seeded over a glass coverslip placed inside a 35mm petri dish or in the PDMS support, previously prepared. This procedure is conducted under a biosafety cabinet to ensure sterility and minimize contamination risk.

First, culture media and Trypsin/EDTA solution (Sial) are warmed in a 37°C waterbath (Bagnomaria Serie Pura, Julabo, Sacco srl). Trypsin is a proteolytic enzyme used to cleave adhesion proteins, allowing cells to detach from the flask surface. Next, the flask containing the cells, is taken from the incubator and placed in the biosafety cabinet. The splitting protocol can then begin:

- The spent medium (8-9 mL) is removed by aspiration with a serological pipette. Approximately 6 mL are stored in a 15 mL Falcon tube, and the rest is discharged.
- The bottom surface of the flask is gently rinsed with 5-7 mL of Phosphate Buffer Saline (PBS, Sial) at room temperature to remove any trace of culture media containing trypsin inhibitors. Then PBS is then aspirated and discarded.
- 3 mL of Trypsin are added to cell layer, and the flask is placed in the incubator for 3-4 minutes. The detachment of cells can be observed by gently tapping the flask.
- The 6 mL of stored medium are added to the flask to inhibit the action of Trypsin. The magnesium (Mg) and calcium (Ca^{2+}) ions in the cell media inhibit Trypsin by affecting its ability to break down the proteins of the cells. The cells are then aspirated from the bottom surface and transferred to the Falcon tube. The Falcon tube is centrifuged at 1500 rpm and 25°C for 5-10 min with soft deceleration. This process induces sedimentation, forming a dense pellet of cells, while the residues remain in the supernatant. The liquid is aspired and discarded.
- The cells are counted, and an appropriate aliquot of cell suspension is added to a new 75 cm² flask, with 8-9 mL of fresh culture media. An inoculum of $2 \cdot 10^3$ to $6 \cdot 10^3$ cells/mm² is recommended.

The splitting is usually performed every 3-4 days, but it can be done earlier if necessary. The color of the medium indicates cell growth, shifting from red to yellow due to the pH change from nutrient consumption.

2.2.3 Cellular counting procedure

The cell counting procedure is crucial for determining the total number of cells suspended in a given volume of liquid. This step is essential because cells must be seeded at a precise density to ensure that each experimental sample contains the correct number of cells, making the experimental results comparable. The commonly used tool for this procedure is the Bürker chamber (Figure 2.9a), a rectangular slide with a recessed area that forms a small chamber. Specifically, the Bürker chamber features two 3 x 3 mm squares, each with a depth of 0.1 mm. Each of these squares is divided into 9 smaller squares (1 mm each), which are further subdivided into 16 even smaller squares (0.2 mm each). The main steps for cell counting are as follows:

- The cell suspension is diluted with fresh culture media and gently pipetted to avoid cell sedimentation and clustering.
- 10 μ L of the cell suspension is collected with a micropipette and placed in a clean Falcon tube.
- 10 μ L of Trypan Blue (Invitrogen) is then added to the cell suspension. Trypan Blue is a dye that stains only dead cells blue, allowing for the identification and counting of viable (living) cells (see Figure 2.9b).

After gently mixing the cell suspension with Trypan Blue to ensure an even distribution of the dye, 10 μ L of the stained cell suspension is collected with a micropipette. This volume is then placed on one side of the Bürker chamber, forming a bubble that fills the chamber.

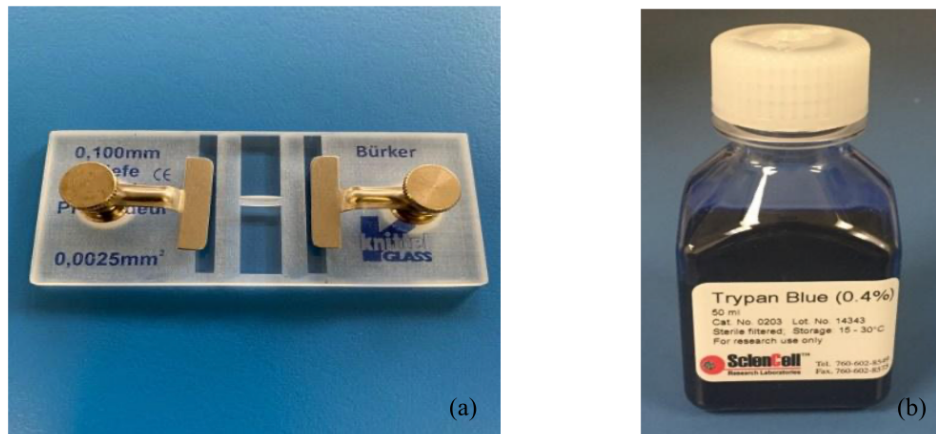


Figure 2.9 Bürker chamber and trypan blue to perform the cellular counting procedure. **a.** Bürker chamber used in the laboratory. **b.** Trypan blue (0.4%).

The chamber is observed under a microscope using a 10x magnification (Fig. 2.10). At least three principal squares are counted, and the number of cells in each square is recorded.

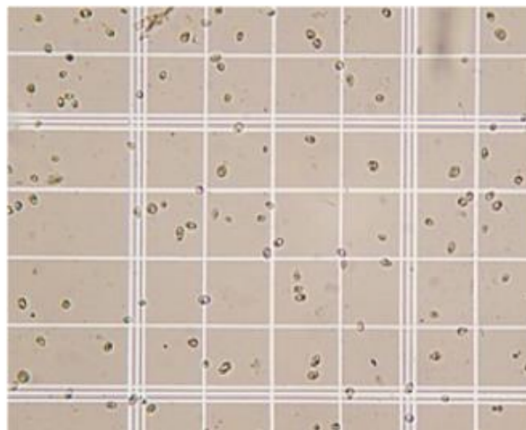


Figure 2.10 Representative image of one of the nine squares of the Bürker chamber.

To calculate the total number of cells, the following equation (2.3) is used:

$$Cell_{tot} = \bar{M} * 2 * 10^4 * V_{tot}, \quad (2.3)$$

where $\bar{M}$ is the average number of cells counted in the three (or more) squares of the Bürker chamber; 2 is the dilution factor for Trypan Blue; 10^4 is a correction factor related to the geometry of the hemocytometer and V_{tot} is the total volume of medium and cells (mL) in the Falcon tube.

2.2.4 Preparation of the petri dish and cells seeding

To conduct the experiments using the electric and magnetic field generators, cells must be seeded on an 18 mm diameter coverslip, inside a 35 mm petri dish. This specific configuration is chosen because it allows for the proper application of the two experimental setups. Instead of seeding cells directly onto the petri dish surface, coverslips are used to facilitate sample extraction after the experiment. This approach enables subsequent analysis using microscopy and other assays while also minimizing the volume of reagents required. In fact, the coil is designed to match the dimensions of the petri dish, allowing the setup to be placed inside it. A flask, due to its larger size, would not fit within the coil or capacitor, making the use of coverslips within the petri dish the most suitable option. Before placing the coverslip inside the petri dish, it must be sterilized in ethanol at 70% and then rinsed with milli-Q water. Following this procedure, the coverslip is sterilized again using UV rays in a laminar flow cabinet and allowed to air dry completely for at least 30 minutes before cell seeding.

After sterilization, the coverslip is placed inside the petri dish with the aid of a drop of PBS to ensure it adheres to the petri bottom. Cells are then seeded at a density of approximately 125×10^3 cells per coverslip.

If the PDMS support described in §2.1.3 is used, three coverslips of 12 mm in diameter are placed inside the support. The protocol remains the same. The cells are seeded at a density of 100×10^3 cells per coverslip.

2.2.5 Protocol for cell fixation

To preserve cellular structures and biomolecules while halting biological activity, cells are fixed using 4% paraformaldehyde (PFA, Sigma-Aldrich). As a cross-linking agent, PFA penetrates the cell membrane and interacts with proteins, DNA, and other cellular components, effectively stabilizing them. This concentration is selected for its ability to achieve efficient fixation while minimizing structural distortion or damage.

For 2D cell cultures, after removing the culture medium and washing with PBS, fixation is performed by incubating the cells in a pre-prepared 4% PFA solution in PBS for 15 minutes at room temperature. It is crucial that solution properly covers the coverslip.

Following fixation, cells undergo three washes with PBS, each lasting 5 minutes, to remove residual fixative.

At this stage, the fixed cells are ready for subsequent analyses, such as immunostaining and fluorescence microscopy, enabling the examination of cellular morphology and molecular organization.

2.2.6 Phalloidin and DAPI staining protocol

To study the morphological changes in cells that occur after exposure, a staining protocol with phalloidin and DAPI is employed. Phalloidin (Proteintech), a phallotoxin produced by death cap mushroom *Amanita phalloides*, is used in fluorescent microscopy to identify actin filaments, a protein which constitutes the cell's cytoskeleton. Phalloidin specifically binds to F-actin, enabling visualization of the cytoskeleton and its arrangement, which is fundamental for understanding cell shape, migration, and interactions. In parallel, DAPI (Life Technologies), short for 4',6-diamidino-2-phenylindole, is a fluorescent stain that strongly binds to adenine-thymine (AT) rich regions of DNA, emitting blue fluorescence upon excitation with ultraviolet light. This important property makes it a valuable tool for assessing nuclear structural integrity and overall cell viability. The protocol for staining with Phalloidin and DAPI markers begin with the previously described fixation in PFA. First, Tryton at 0.1% is added to facilitate the entry of the fluorescent dyes. Phalloidin is then diluted in PBS at a ratio of 1:200 and incubated for 40 minutes at room temperature. Subsequently, DAPI is added at a concentration of 1:1000 and incubated for an additional 15 minutes. Finally, the cells undergo three washes with PBS, each lasting 5 minutes.

2.2.7 MTT assay

The MTT assay was performed to evaluate cell viability following exposure to the experimental conditions. This colorimetric assay is based on the ability of viable cells to reduce the MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Roche) into insoluble formazan crystals that have a purple color, which can be solubilized and quantified spectrophotometrically.

Before the treatment, the culture media of the cells is replaced with 100 μ L of fresh media in order to obtain the volume needed for the application of the protocol. Then the cells are exposed to the treatment.

Following the treatment, is added to the cells 10 μ L of MTT reagent and let it incubate for 4 hours at 37°C to allow formazan crystal formation.

After incubation, the culture medium containing the crystals is transferred to a 96-well plate, and 100 μ L of solubilization buffer (DMSO, Sigma-Aldrich) is added to each well to dissolve the formazan crystals. Subsequently, the plate is incubated overnight to ensure complete dissolution of the crystals.

Typically, this protocol is performed directly in a 96-well plate. However, in this specific study, this was not possible because the 96-well plate is incompatible with the two experimental setups described in §2.1.1 and §2.1.2. For this reason, the first steps had to be carried out directly in the petri dish, with the transfer occurring only after formazan crystal formation and before the addition of DMSO. After the overnight incubation, the absorbance of each well - dependent on the quantity of formazan crystal formed - is measured using a microplate reader, specifically the Wallace Victor2 model 1420 multilabel counter (PerkinElmer), at 560 nm, with a reference wavelength of 630 nm to correct for background absorbance. To evaluate cell viability, the absorbance value measured by the plate reader is divided by the average absorbance of the control samples specific to each cell type.

2.2.8 Immunofluorescence staining and MFI quantification

Immunofluorescence staining is a technique that utilizes the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell. There are two types of immunofluorescent techniques: primary (or direct) and secondary (or indirect). The direct technique uses a single primary antibody, chemically linked with a fluorophore which is a fluorescent chemical compound that can re-emit light upon light excitation. The indirect technique, employed in this protocol, uses two antibodies: the primary antibody specifically binds the target molecule and the secondary antibody, which carries the fluorophore, recognizes and binds to the primary antibody. This provides signal amplification by increasing the number of fluorophore molecules per antigen.

In this experiment, Ki-67 was used as a marker of cell proliferation. The Ki-67 protein (also known as MKI67) is a cellular marker for proliferation. Ki-67 is present during all active phases of the cell cycle (G1, S, G2 and M), but is absent in resting cells (G0).

Cellular content of Ki-67 protein markedly increases during cell progression through S phase of the cell cycle. Therefore, the nuclear expression of Ki-67 can be evaluated to assess tumor proliferation by immunohistochemistry. A primary anti-Ki67 polyclonal rabbit antibody was used (Proteintech), followed by a secondary CoraLite488-conjugated Goat Anti-Rabbit IgG (Proteintech).

After the fixation protocol, the coverslips with the treated cells, are transferred on a microscopy glass slide

- Using a PAP pen, an unbroken circle was drawn around the coverslip containing the fixed cells to create a hydrophobic barrier, and the wax was left to dry for 15–30 minutes.
- The cells are rehydrated by washing the coverslips three times with phosphate-buffered saline (PBS) for 5 minutes each.
- The samples are incubated in 0.1% Triton X-100 in PBS for 15 minutes at room temperature and then washed once with PBS for 5 minutes.
- The samples are then blocked for 1 hour at RT in a blocking solution containing 5% bovine serum albumin (BSA, Sigma Aldrich) and 0.1% Triton X-100 in PBS. (Blocking solution preparation: 2.5 g of BSA is dissolved in 50 mL of PBS, and 50 μ L of Triton X-100 is added).
- The primary antibody (anti-Ki67 polyclonal rabbit antibody, Proteintech) is diluted 1:200 in blocking solution and added to the samples, which are then incubated overnight at 4°C. The samples must be kept in a humidified chamber during incubation to prevent them from drying out.
- The samples are washed twice with 0.1% Triton X-100 in PBS for 30 minutes to reduce background fluorescence, followed by an additional wash with PBS for 30 minutes.
- The samples are then incubated with the secondary antibody (CoraLite488-conjugated Goat Anti-Rabbit IgG (Proteintech)) diluted 1:800 in blocking solution for 1 hour at room temperature. Due to the presence of the fluorophore, the samples must be protected from light by covering them with aluminium foil.
- A wash is performed with PBS for 5 minutes, followed by incubation with 1:1000 DAPI in PBS for 15 minutes. The samples are then washed three times with PBS for 5 minutes each.

- One drop of 50% glycerol is applied onto the sample, and the coverslip is placed.

Finally, samples were analysed with a confocal fluorescence microscope. Analyses on acquired images were performed using ImageJ. This allows to evaluate data collected on a microscope by quantitating the relative levels of a molecule of interest by measuring its mean fluorescent intensity (MFI) across a region of interest (ROI). The cell samples were observed with a 63× objective and three different dyes were set: ATTO488 for green channel (Antibodies) and DAPI for blue channel (cell nuclei). Prior to images acquisition, some parameter must be properly adjusted. The laser intensity must be as low as possible to minimize photobleaching and saturation of fluorophores and at the same time maintain an adequate signal-to-noise ratio for image analysis. This can depend on the properties of the fluorophore, objective, laser wavelength, age of the laser, and wattage. For quantitation, it is essential that the laser power be kept constant across the analysis and it is recommended to leave the laser power at 2% or below. Another parameter to adjust is the pinhole diameter, often expressed in Airy Units (AU) which are defined as $1 \text{ Airy Unit} = (0.61 \times \text{wavelength of light} \times \text{total Magnification}) / \text{Numerical aperture of the lens used for imaging}$. The recommended setting for the pinhole is usually 1 AU. The Master gain and the digital gain are other adjustments that set the detection threshold and the dynamic range of the detectors which are key for reliable quantitation¹⁴. The first setting is ‘Gain (Master)’: this setting adjusts the sensitivity of the detector, however, increasing the sensitivity can also increase the noise. It is recommended to not go above 800–850 for gain master. ‘Digital Gain’: adjusting this setting reamplifies intensities from master-gained images. This amplifies the signal at the same rate as it amplifies noise. It is recommended to leave this value set to 114.

For the quantitation of images using ImageJ, each image must be converted in 16 bit and the fluorescence intensity measured keeping constant the threshold for all the data collected for every antibody staining. Thresholding is a technique for segmenting an image into two classes of pixels, typically called “foreground” and “background”. It works by choosing a value cutoff, thus every pixel less than that value is the background, while every pixel greater than that value is considered foreground. After the MFI calculation it is preferable to subtract the background. This step consists in selecting and measuring a non-fluorescent area of the same image or a negative control prepared during

the same experiment and imaged under the same confocal parameters as the image being quantitated. MFI of that non-fluorescent area/negative control image is then subtracted from MFI.

2.2.9 pH measurement

To assess pH variations following exposure to the two fields, a pH meter (Hanna instruments) is used. Before measurement, the pH electrode is rinsed with Milli-Q water to prevent contamination in the measure. After exposure, the culture medium in each petri dish is collected and transferred into a 50 mL falcon tube. The pH meter is then turned on and calibrated according to the manufacturer's instructions. The pH measurements is performed by immersing the electrode directly into the Falcon tube containing the exposed medium. The measured pH value is then displayed on the screen.

2.3 Statistical Analysis

The statistical analysis was performed with GraphPad Prism software. All data in graphs are from at least three independent experiments. Statistical significance was determined using unpaired Student's t-test or ANOVA analysis. Asterisks indicate a significant difference between the treated and the control group, unless otherwise specified (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Chapter 3

Results

This section presents the results of Comsol Multiphysics simulations verifying the setup's functionality, including electric and magnetic field analyses. Additional simulations were performed to assess the impact of a PDMS support on field parameters. The biological experiments evaluating field-induced effects are also discussed, first for the electric field and then for the magnetic field.

3.1 Results of the COMSOL Multiphysics® simulations

In this section, the results obtained from the Comsol simulation will be analyzed. The goal is to verify that the designed setup effectively generates the desired intensities and frequencies in the petri dishes, ensuring proper exposure of the cells.

3.1.1 Results of the numerical Comsol simulation for the electric field setup

In the previous chapter, the electric field setup was explained (§2.1.1). The goal is to verify that the desired electric field value which of 1 V/cm is achieved in the petri dish containing the cells. The results of the analysis are shown in Figure 3.1.

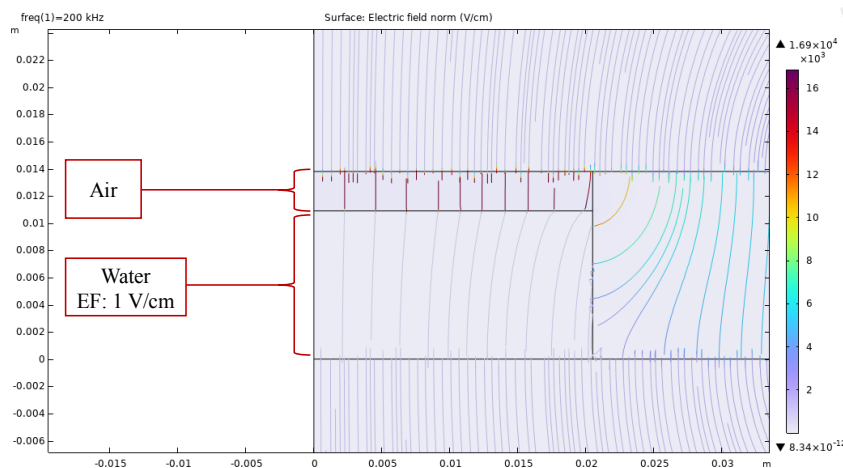


Figure 3.1 Electric field distribution in the experimental setup. The figure illustrates the electric flux density around the petri dish. The color gradient represents different electric field (EF) intensities, with the highest value in the air at the top.

In the color bar, the intensity values of the electric field to which the petri with the cells is exposed are reported. As stated in § 2.1.4.2, due to the lower relative permittivity of air, which allows the electric field to pass through more easily, the electric field intensity is higher in the air inside the petri dish. This is evident from the dark red color of the field lines in the image.

On the other hand, in water, which has a higher permittivity, the electric field intensity is significantly lower. This is observed in the blue-colored of the field lines, indicating an electric field of 1 V/cm. This analysis confirms the initial hypothesis: the highest electric field intensity is present in the air, while in the water, the desired intensity of 1 V/cm is achieved. This verification confirms that the designed setup functions as intended.

3.1.2 Results of the numerical Comsol simulation for the magnetic field setup

In Chapter 2 (§2.1.2), the setup for the magnetic field was also explained. This simulation aims to demonstrate that the designed setup functions as required. The results of the simulations are reported in Figure 3.2.

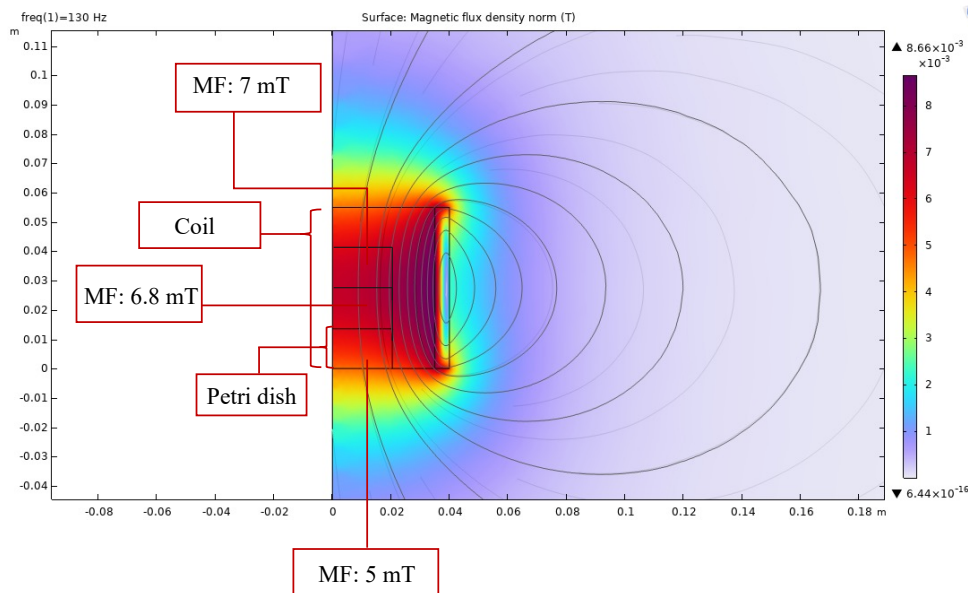


Figure 3.2 Magnetic Field Distribution in the Experimental Setup. The figure illustrates the magnetic flux density around the coil at a frequency range of 1–130 Hz. The color gradient represents different magnetic field (MF) intensities, with the highest value (7 mT) at the top of the coil and decreasing to 5 mT at the bottom.

The color bar displays the magnetic field values. As shown in Figure 3.2, the three petri dishes receive slightly different magnetic field intensities due to their positions inside the coil. The intensity depends on the density of the copper turns.

At the top and at the center of the coil, the intensity is highest because the coil turns are most concentrated in this region. Meanwhile, at the bottom, as observed in the image, the color shifts toward yellow, indicating a lower magnetic field intensity.

In the experiments, three petri dishes will be used, stacked one above the other, as shown in Figure 3.2. From the simulation, it can be seen that the petri dish at the bottom of the coil receives 5 mT, represented by an orange color. The middle petri dish is exposed to 6.8 mT, displaying a lighter red shade. This intensity is higher than that at the bottom but lower than at the top. Specifically, the petri dish at the highest level- positioned above the other two but not at the very top of the coil - is subjected to a magnetic field of 7 mT, as indicated by the darker red color in the simulation.

3.1.3 Results of the numerical Comsol simulation for the electric field setup with the PDMS support

In the experimental setup, a PDMS support has been added to increase the number of tests per experiment and to allow exposure of different cell types to two distinct magnetic field intensities. Therefore, it is important to verify that this support does not alter the electric field parameters to which the petri dishes with cells are exposed.

The Comsol simulation results are shown d in Figure 3.3.

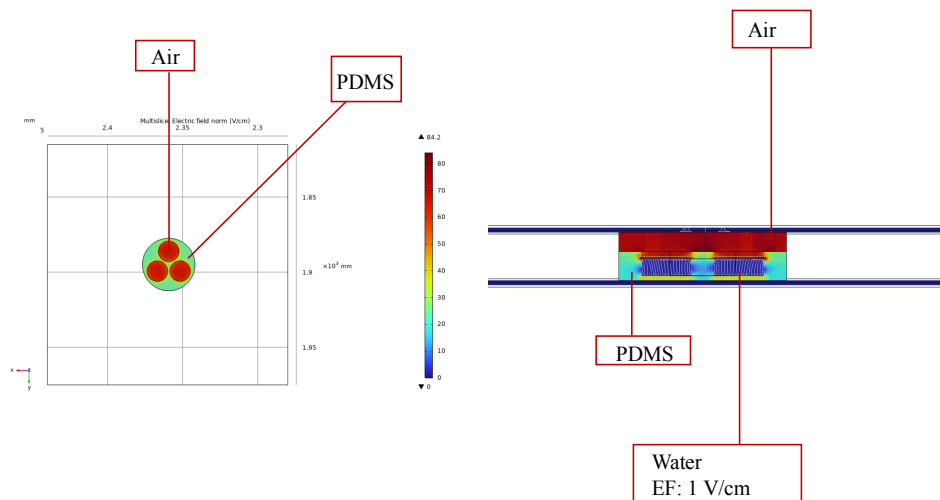


Figure 3.3 Electric field distribution in the experimental setup. The figure presents the electric field distribution within the experimental setup, illustrating the effects of the PDMS support. The distribution of the field within the structure is depicted using a color gradient, showing areas of higher and lower intensity. The PDMS support is positioned between the electrodes, ensuring that the field parameters remain within the desired range for cell exposure.

The figure shows that the highest intensity of the electric field is concentrated in the air, represented by the red color, while the field intensity is significantly lower in the PDMS, appearing light green. In the water, the simulation displays a dark blue color, indicating an electric field of 1 V/cm.

This simulation confirms that, under the same operating conditions, the electric field reaching the petri dish with the cells remains unchanged at 1 V/cm.

3.1.4 Results of the numerical Comsol simulation for the magnetic field setup with the PDMS support

As previously explained, the PDMS support has also been implemented in experiments using the magnetic field. The goal of this simulation is to determine whether the support alters the field parameters. Figure 3.4 shows the results of the Comsol simulation.

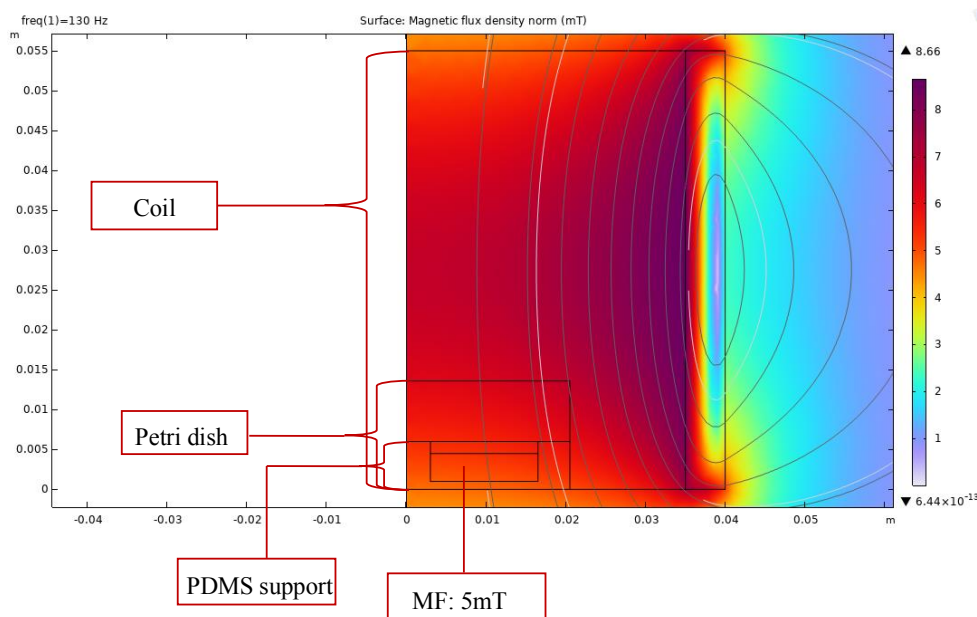


Figure 3.4 Magnetic field distribution in the experimental setup. The figure presents the magnetic field distribution within the experimental setup, illustrating the effects of the PDMS support. The distribution of the field within the structure is depicted using a color gradient, showing areas of higher and lower intensity. The PDMS support is positioned inside the coil, ensuring that the field parameters remain within the desired range for cell exposure.

For simplicity, only one petri dish is shown in Figure 3.4; however, the actual setup consists of three petri dishes stacked one above the other, as explained in §3.1.2.

As observed in the simulation, the magnetic field to which the petri dish is exposed remains at 5 mT, even in the presence of the PDMS. This is indicated by the light red color at the bottom of the petri dish, where the cells will be located. The magnetic field intensity at the bottom remains consistent with the simulation conducted without the PDMS support. Similarly, the magnetic field intensities remain unchanged for the other two petri dishes remain unchanged.

3.2 Biological validation: effects of the electric field

This section will analyze and discuss the biological effects induced by exposure to electric fields. First, the effects of the electric field will be evaluated, focusing on morphological changes. Then, to assess cell viability and proliferation, the results from the MTT assay and Ki-67 staining will be examined. Finally, since a color change in the culture medium was observed during the tests, the results of pH measurements and analysis will be reported.

As previously explained (§2.1.1.1) the experiments involving the electric field were conducted with an intensity of 1 V/cm, a frequency of 200kHz and an exposure time of 24 hours. The effects were investigated immediately after exposure, as well as 24 hours and 48 hours later, for both the U-87 MG and astrocyte cell lines.

3.2.1 Morphological changes in brightfield imaging

First, an analysis of images taken using brightfield microscopy will be presented, focusing on the inhibition of cell growth and preliminary observations of morphological changes after exposure to the electric field in both U-87 MG cells and astrocytes.

3.2.1.1 U-87 MG cell line

Before exposure, the glioblastoma (U-87 MG) cells in the control condition exhibit a stretched and stellate morphology, resembling epithelial-like fibroblasts. They are well-adhered to the coverslip substrate and tend to form aggregates. Figure 3.5 shows them under bright field microscopy at different magnifications.

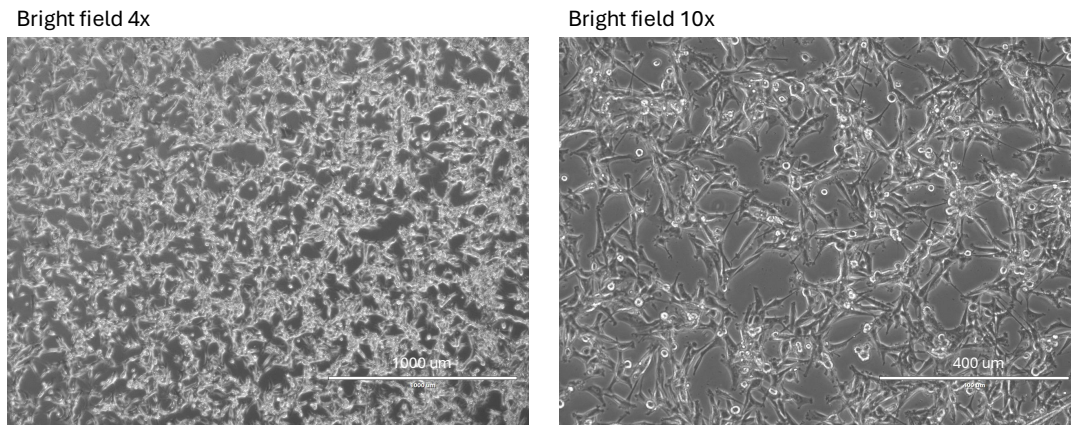


Figure 3.5 Brightfield imaged of U-87 MG cells. Images at different magnification for the control condition for U-87 MG cells before exposure.

In Figure 3.6 are shown the glioblastoma U-87 cells immediately after the exposure to the electric field for 24 hours.

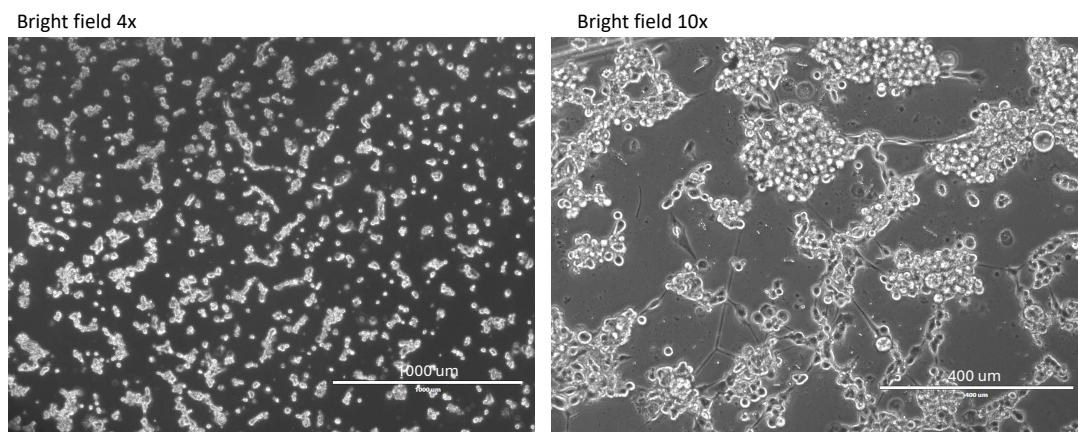


Figure 3.6 Brightfield imaged of U-87 MG cells. Images at different magnification for the U-87 MG cells immediately after the 24 hours exposure of the electric field.

After exposure, the cells exhibit an altered morphology, becoming rounder, and their number significantly decreases as many detach from the coverslip.

Then, the morphology and visible signs of cell recovery are analyzed under the microscope 24 hours after electric field exposure. Figure 3.7 shows the appearance of the cells at this time point, where they appear to be gradually reassuming their usual stellate shape. In fact, the cells appear more elongated compared to those in Figure 3.6, and their

number seems to be increasing due to the reattachment of previously detached cells to the surface.

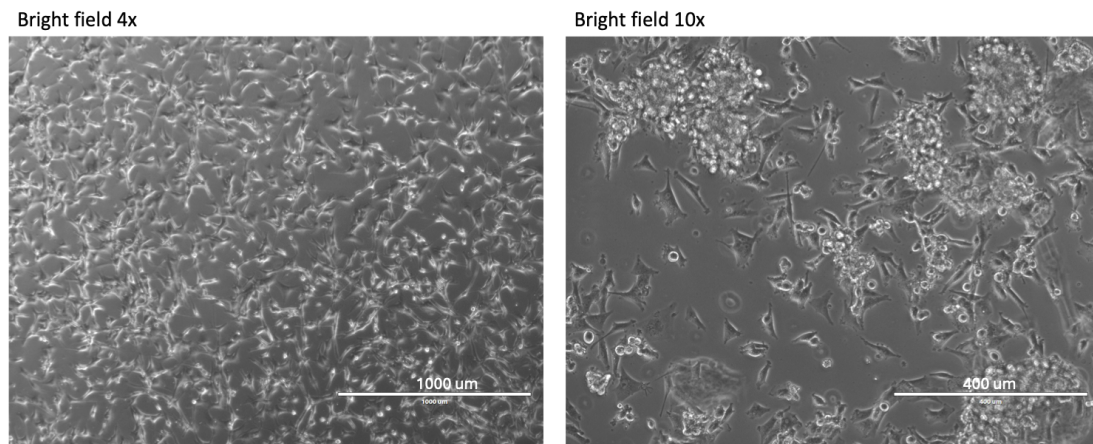


Figure 3.7 Brightfield imaged of U-87 MG cells. Images at different magnification for the U-87 MG cells 24 hours after the end of the electric field exposure.

Furthermore, for the same purpose as before, the cells are observed 48 hours after exposure to the electric field to assess further changes and signs of recovery.

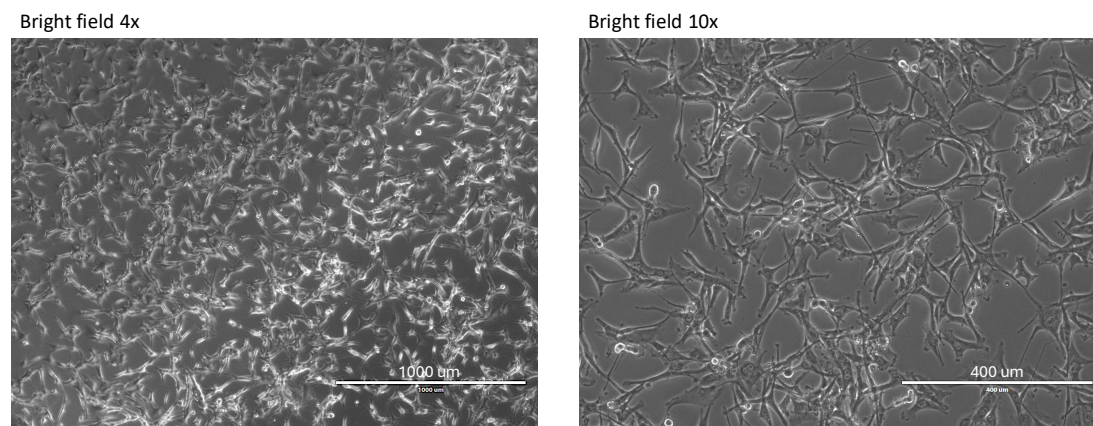


Figure 3.8 Brightfield imaged of U-87 MG cells. Images at different magnification for the U-87 MG cells 48 hours after the end of the electric field exposure.

As shown in figure 3.8, after 48 hours, the cells appear to have fully regained their original shape, as they were before exposure to the electric field. The number of cells seems to increase with recovery time.

3.2.1.2 Astrocytes cell line

In Figure 3.9, astrocyte cells before exposure to the electric field are shown.

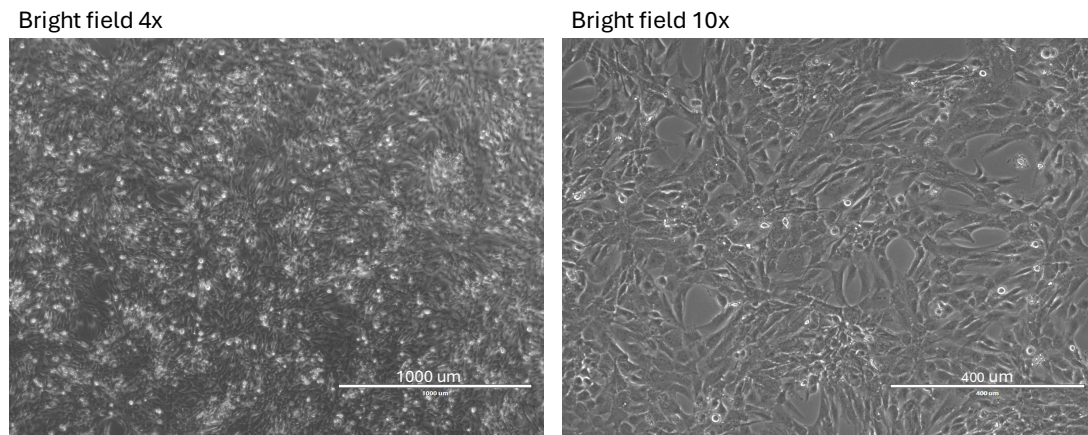


Figure 3.9 Brightfield imaged of Astrocyte cells. Images at different magnification for the control condition for Astrocyte cells before exposure.

As stated by Baldwin et al. (2024) and supported by the reported images, these cells exhibit a characteristic stellate morphology with multiple short cytoplasmic extensions. They appear well-adhered to the substrate and do not form aggregates, maintaining a flatter distribution compared to U-87 MG cells. In Figure 3.10, the cells immediately after electric field exposure are shown.

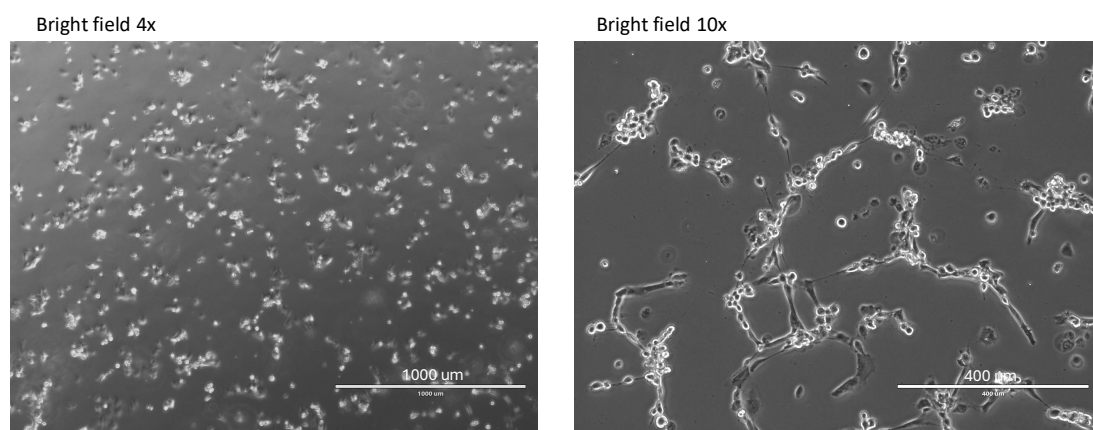


Figure 3.10 Brightfield imaged of Astrocyte cells. Images at different magnification for the astrocyte cells immediately after the 24 hours exposure of the electric field.

It can be observed that their morphology changes significantly after the exposure. They assume a rounder shape, and some cells detach from the coverslip substrate. Their number is also severely reduced.

In Figure 3.11, astrocyte cells are shown 24 hours after electric field exposure.

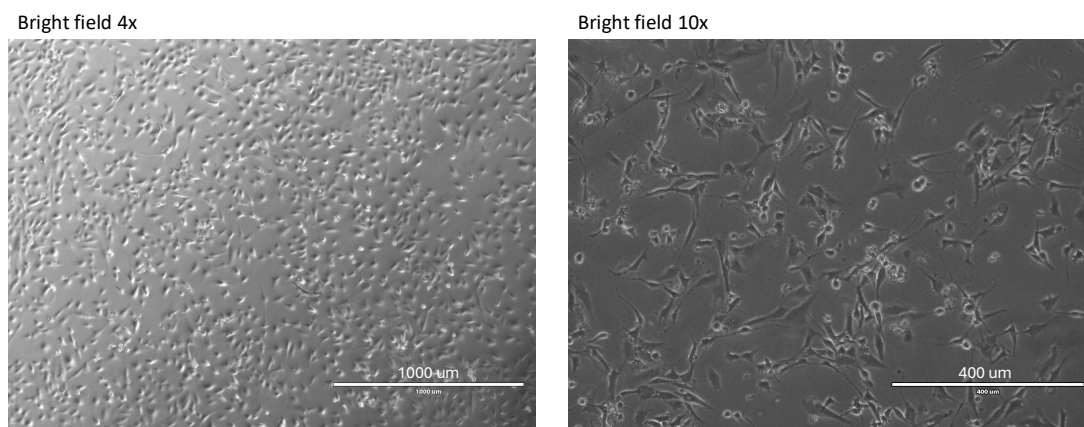


Figure 3.11 Brightfield imaged of Astrocyte cells. Images at different magnification for the Astrocyte cells 24 hours after the end of the electric field exposure.

It is noticeable that the cells have recovered, regained a stellate-like shape, and increased in number. This increase may also be due to some detached cells reattaching to the substrate. Then, the effects after 48 hours were also analysed and are shown in Figure 3.12.

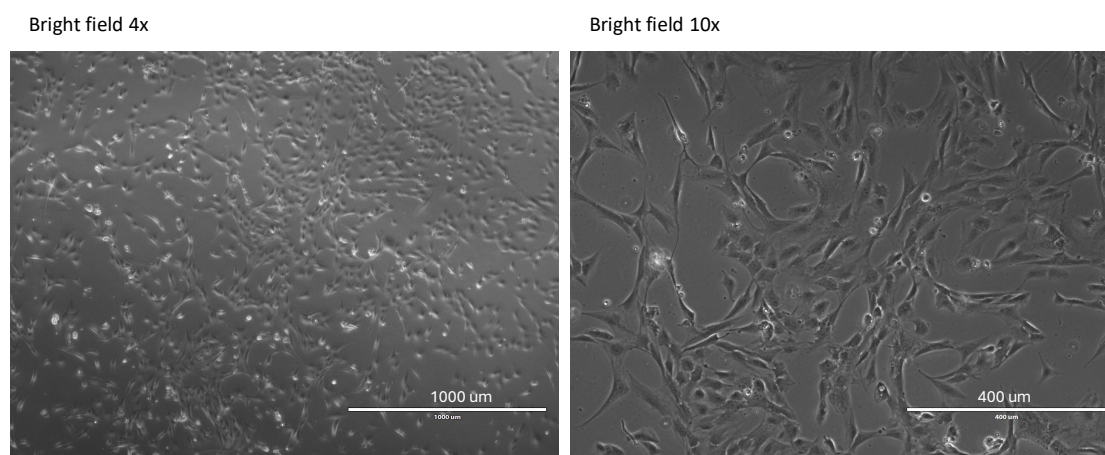


Figure 3.12 Brightfield imaged of Astrocyte cells. Images at different magnification for the Astrocyte cells 48 hours after the end of the electric field exposure.

After 48 hours, the cells recovered, and their shape was very similar to the one observed before the exposure.

3.2.2 Morphology changes observed with phalloidin and DAPI staining

In this section, the results of phalloidin and DAPI staining are reported. The time points analyzed are the same as before: immediately after exposure, 24 hours after exposure, and finally 48 hours after exposure for both the cell lines.

3.2.2.1. U-87 MG cell line

Figure 3.13 illustrate the results of phalloidin and DAPI staining on the U-87 MG cells before the treatment.

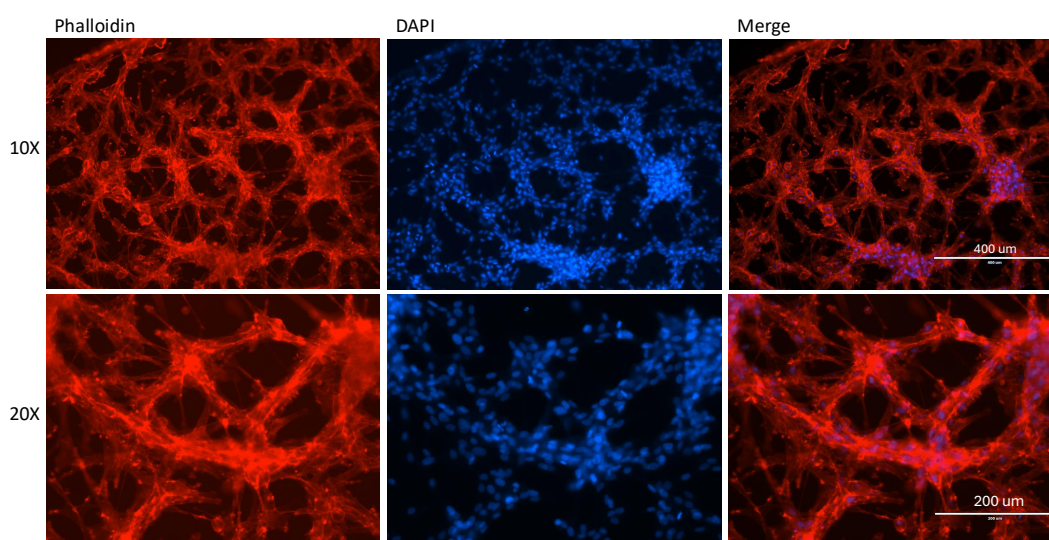


Figure 3.13 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells before treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10×) and 200 μm (20×).

As observed in the bright-field images, the cells exhibit an elongated shape, highlighted by F-actin extensions, which are key components of the cytoskeleton. The cytoskeleton provides structural support, helping the cell maintain its shape. The nuclei, stained in blue, appear numerous and correctly positioned relative to the F-actin structures. The high proliferation rate is indicated by the large number of nuclei and well-developed filamentous actin.

Figure 3.14 presents the results of the staining immediately after exposure to the electric field.

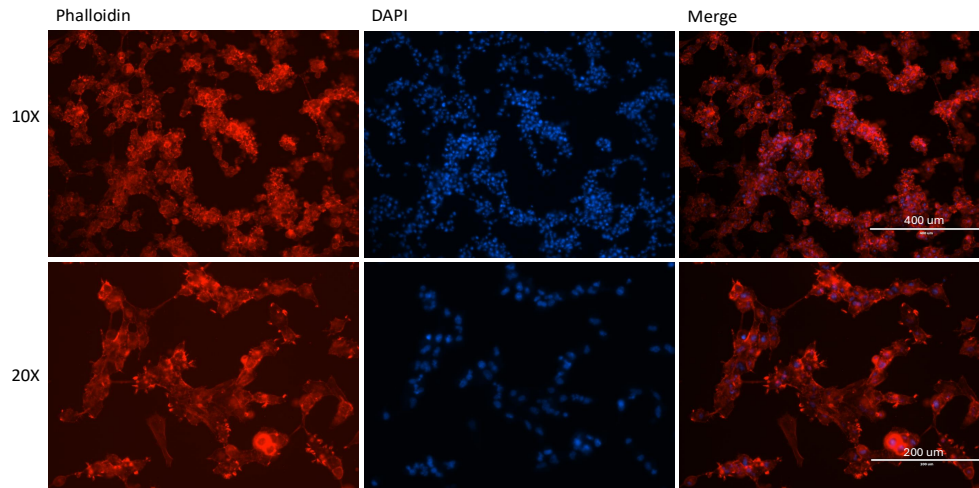


Figure 3.14 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after electric field treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10×) and 200 μm (20×)

The cells appear significantly different from before: the F-actin prolongations are shorter and fragmented. The cells show signs of stress, and the number of nuclei has decreased. As observed and supported by Schlieper-Scherf et al. (2024), after exposure, the cell density is severely reduced, and the electric field also disrupts network interconnectivity and structural integrity. Figure 3.15 presents the results of the staining 24 hours after electric field exposure.

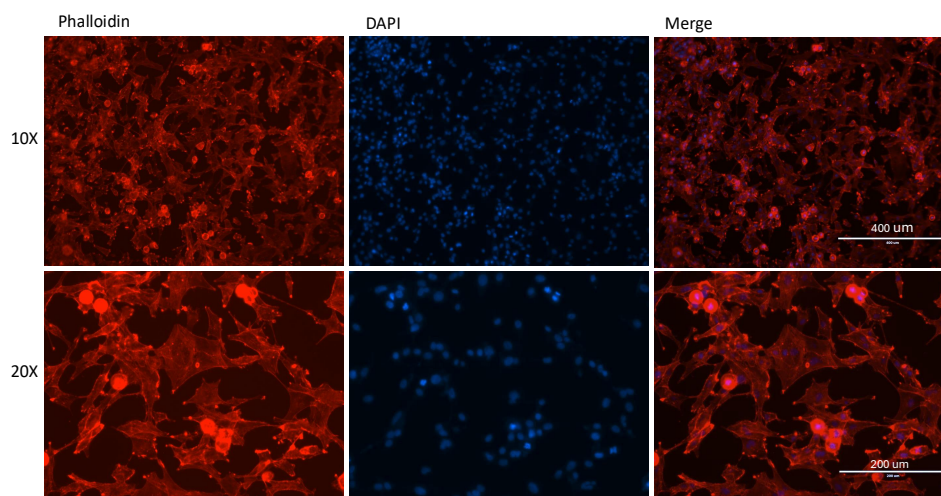


Figure 3.15 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells 24 hours after electric field treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10×) and 200 μm (20×).

The cells appear to have increased in number, as also shown in Figure 3.7 in the bright-field images. However, their morphology is significantly different from that of the cells in the control condition (see Figure 3.13). These differences were not initially evident in the bright-field images, which led to different considerations. Specifically, the F-actin filaments have not regained their usual conformation; instead, they remain fragmented and less organized. The nuclei are positioned correctly but appear more chaotically arranged, consistent with the altered F-actin structure. This is particularly evident in the 20x magnification microscopy images.

Figure 3.16 presents the results of the phalloidin and DAPI staining 48 hours after electric field exposure.

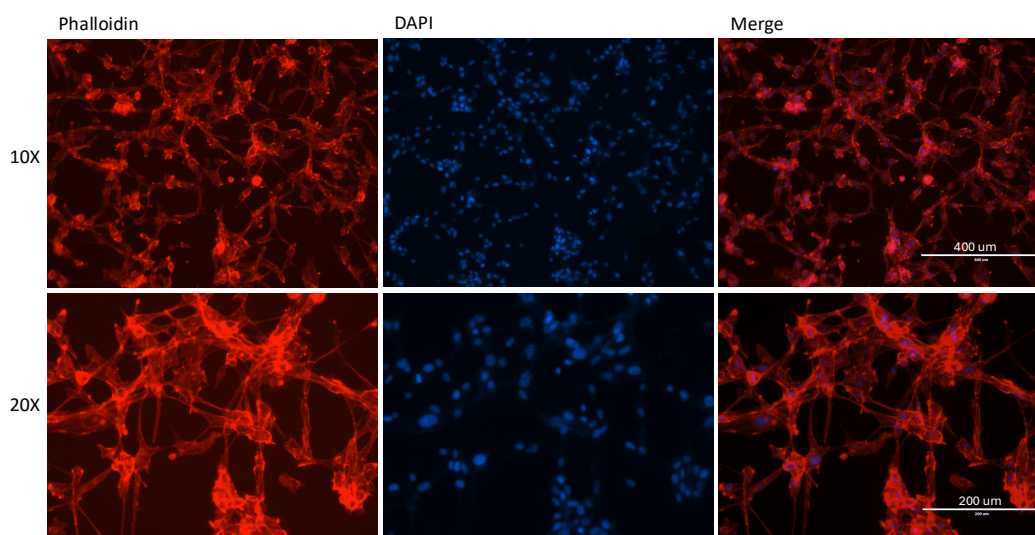


Figure 3.16 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells 48 hours after electric field treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 µm (10×) and 200 µm (20×).

After 48 hours of exposure, the cells have not regained the organization they had before exposure. The F-actin filaments appear less fragmented, and the cells seem to be attempting to assume an elongated conformation; however, their structure appears altered, as the extensions are shorter, thinner, disorganized and less polarized towards a specific direction. Additionally, the nuclei remain chaotically positioned, as they were in Figure 3.15, and are less compact. The damage observed in the cytoskeleton indicates damage to the cellular membrane, which may be responsible for cell death and could also favour the eventual uptake of chemotherapeutic drugs. However, as highlighted by Chang et al.

(2018) and numerous other studies, this effect is reversible, as partially observed in the cells after 24 and 48 hours (Figure 3.16). This suggests that to obtain continuous membrane disruption requires prolonged exposure.

3.2.2.2 Astrocytes cell line

In Figure 3.17, the results of the phalloidin and DAPI staining for the astrocyte cells in the control condition are shown.

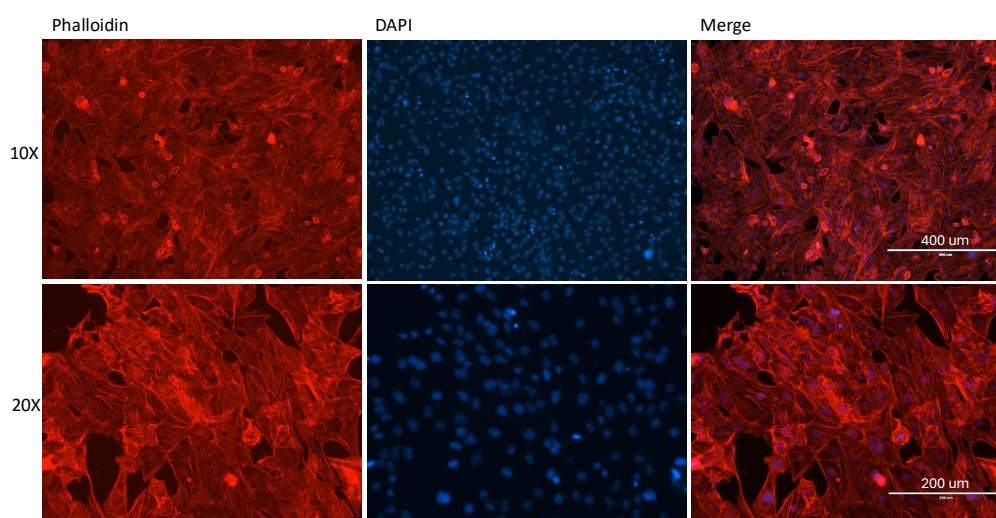


Figure 3.17 Results for Astrocyte cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocyte before treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 µm (10×) and 200 µm (20×).

As observed in the bright-field images, the astrocytes in Figure 3.17 also exhibit short f-actin filaments and are not polarized like the neurons. They are compacted together, showing a “bushy” conformation (as described by Baldwin et al., 2024).

In Figure 3.18, the results of the phalloidin and DAPI staining of the astrocytes after electric field exposure are shown. Exposure to the electric field led to a significant reduction in cell number. The cells appear more fragmented, and the ramification of F-actin filaments is both reduced in number and shorter. The decrease in cell number is further confirmed by the reduced number of visible nuclei compared to Figure 3.17. Moreover, treated cells appear more spread out, less elongated, and less polarized compared to the control cells, suggesting that their adhesion is more evenly distributed

and less oriented in a specific direction, as highlighted and confirmed by Voloshin et al., (2020).

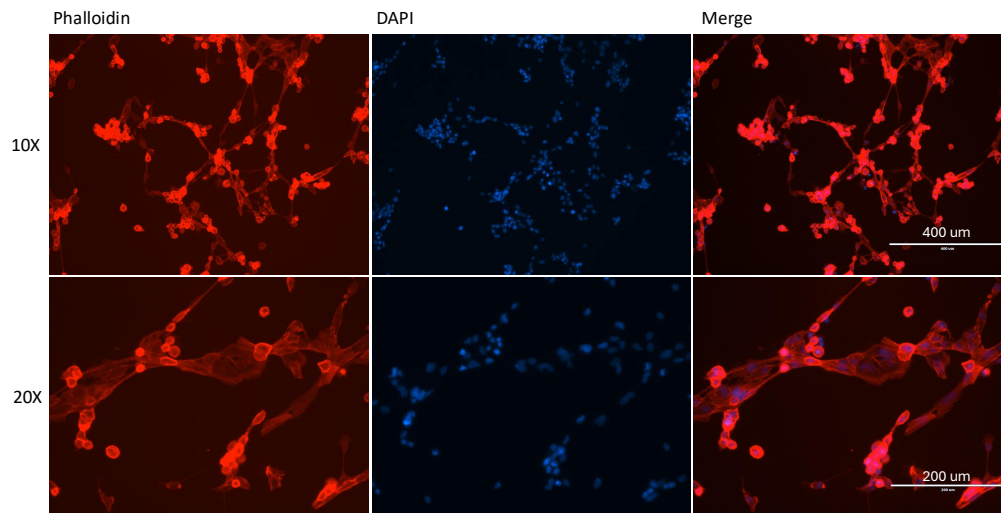


Figure 3.18 Results for Astrocyte cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocyte cells immediately after the treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10×) and 200 μm (20×).

Figure 3.19 shows the results of the phalloidin and DAPI staining for the astrocytes 24 hours after the exposure.

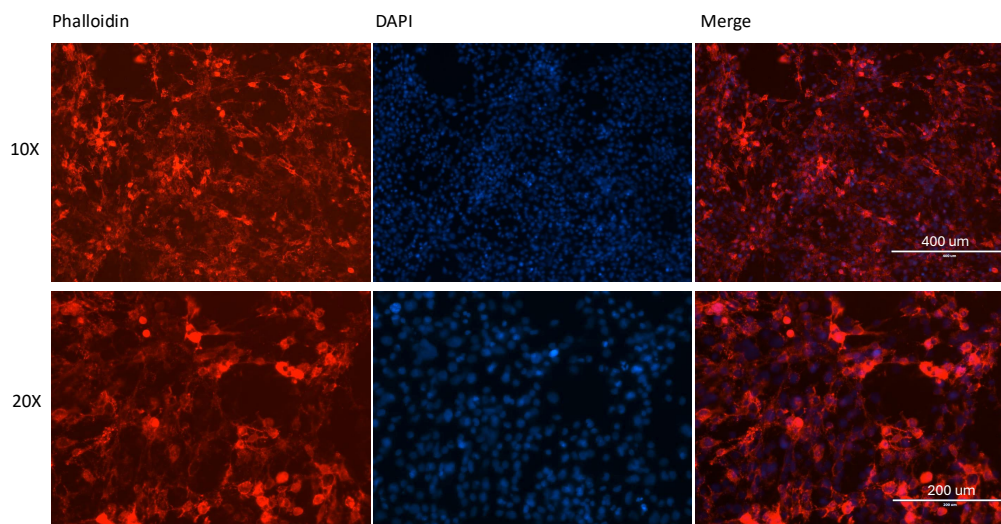


Figure 3.19 Results for Astrocyte cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocyte 24 hours after electric field treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10×) and 200 μm (20×).

The cells have greatly increased in number but have failed to reorganize into the structures they exhibited before exposure. Finally, Figure 3.20 shows the results of the phalloidin and DAPI staining 48 hours after electric field exposure.

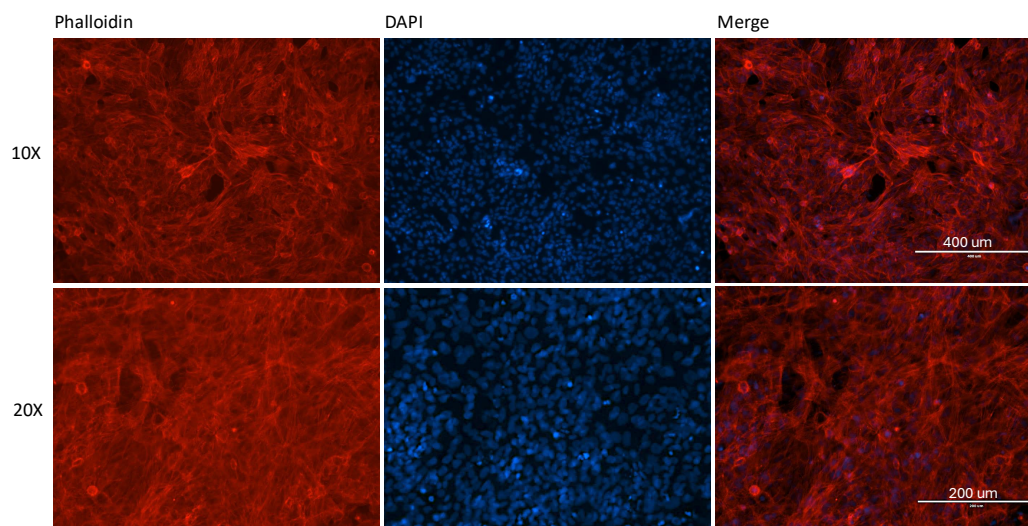


Figure 3.20 Results for Astrocyte cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocyte 48 hours after electric field treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 µm (10×) and 200 µm (20×).

At this time point, the cells appear to have increased in number and become more compact while still exhibiting a disorganized conformation, with shorter and thinner branches. Astrocytes have shown a good capacity to recover from the stress induced by the field.

3.2.3 MTT assay results

In this section, the results of the MTT viability assay are presented. The protocol was applied to two different cell types immediately after electric field exposure.

The results of the MTT assay, presented as a column graph, quantify the viability of U-87 MG cells and astrocytes following electric field exposure.

Statistical analysis was performed using a t-test to compare the treated and control groups.

3.2.3.1. U-87 MG cell line

The MTT results for the U-87 MG cells can be seen in the column graph reported in Figure 3.21.

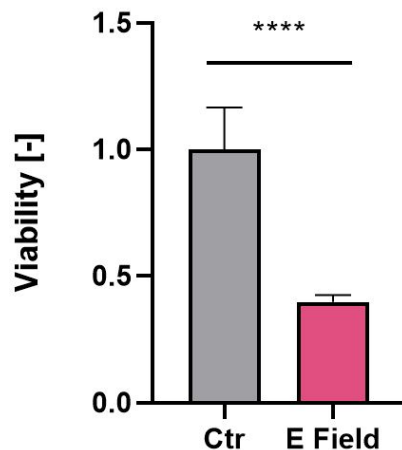


Figure 3.21 Results for the MTT assay for U-87 MG cells. Column plot for the results of MTT assay analysis. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

Cell viability of U87 has significantly decreased compared to the control. The statistical analysis is significant ($p < 0.0001$) meaning that the electric field strongly compromise the viability of the cells. The MTT assay provides an indirect measure of cell viability by assessing metabolic activity, primarily through mitochondrial function. A significant reduction in MTT signal in treated cells compared to controls would indicate decreased viability, potentially due to cell membrane damage, mitochondrial dysfunction, or apoptosis triggered by electric field exposure. Conversely, if no significant difference is observed, this suggests that the cells either recovered rapidly from the stress or that the applied field parameters did not induce substantial cytotoxic effects.

3.2.3.2 Astrocyte cell line

In Figure 3.22. are reported the results for the MTT assay performed on the astrocytes cell line. The data reveal a significant decrease in cell viability after exposure ($p < 0.0001$), indicating that the applied electric field negatively impacts astrocyte viability.

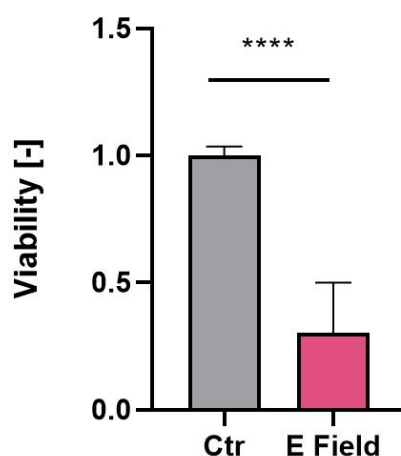


Figure 3.22 Results for the MTT assay for Astrocyte cells. Column plot for the results of MTT assay analysis. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

3.2.4 Immunofluorescence with marker Ki-67: results

The ability of the cells to proliferate after exposure to the electric field is investigated using Ki-67, a proliferation marker. This is assessed by comparing the results of the exposed samples with those of the control. The fluorophore used for Ki-67 is green, meaning that higher green intensity indicates greater cell proliferation. First, the results for U87 MG cells will be presented, followed by the results for astrocytes.

3.2.4.1. U-87 MG cell line

Figure 3.23 shows confocal microscopy images of U-87 MG cells stained with Ki-67, illustrating the results of the staining. It is evident that the cells in the control condition exhibit the highest Ki-67 intensity indicating normal proliferation, as expected. In contrast, the Ki-67 signal is significantly lower in cells exposed to the electric field, suggesting a severe reduction in their proliferative capacity. This may indicate cell death, confirming previous observations.

Additionally, the figure reveals signs of cellular stress in the exposed cells. The nuclei, stained with DAPI, appear irregular in shape and show lower fluorescence intensity compared to the control suggesting a possible disruption of the nuclear membrane.

To further investigate and quantitatively validate these observations, mean fluorescence intensity (MFI) was measured using ImageJ software (Figure 3.24).

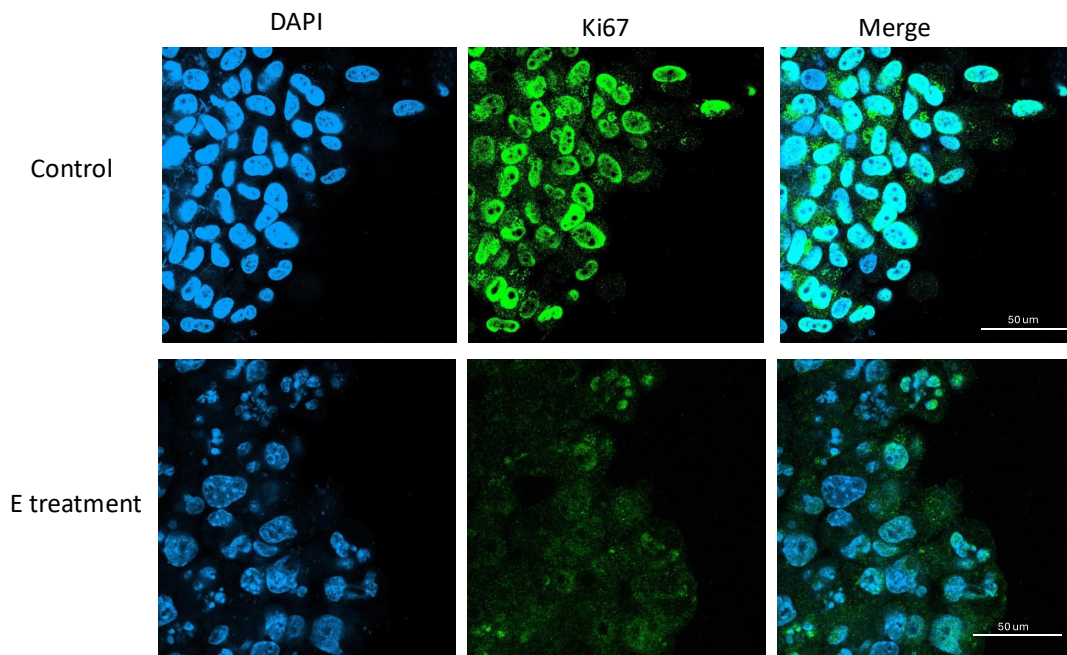


Figure 3.23 Ki-67 staining for proliferation analysis in U-87 MG cells. Representative immunofluorescence images of U87 MG cells under control conditions (top row) and after exposure to the electric field (bottom row). DAPI (blue) stains cell nuclei, while Ki-67 (green) marks proliferating cells. The merged images (right column) show the colocalization of nuclei and proliferation marker expression. Scale bars: 20 μ m.

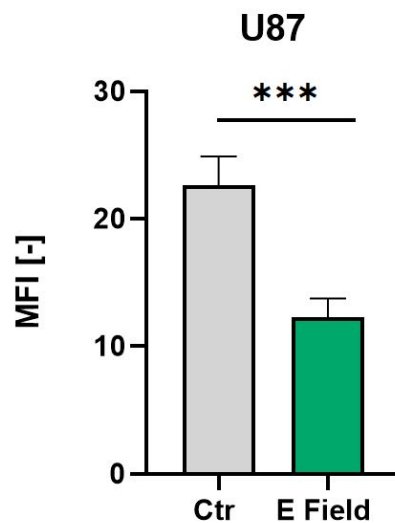


Figure 3.24 Column graph showing the Mean Fluorescence Intensity (MFI) of the Ki67 marker on U87 MG cells for quantification. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

Figure 3.24 presents a column graph showing the mean fluorescence intensity (MFI) calculated for control and exposed U-87 MG cells. The MFI of the control cells is significantly higher than that of the exposed cells, consistent with the confocal images (see Figure 3.23).

Moreover, the analysis is statistically significant ($p < 0.001$), confirming that the decrease in MFI - and consequently, the reduction in U-87 MG cell proliferation is directly caused by exposure to the electric field.

3.2.4.2 Astrocytes cell line

Consequently, results of immunofluorescence staining with the Ki-67 marker for the astrocyte cells are presented.

Figure 3.25 shows confocal microscopy images of stained astrocyte cells.

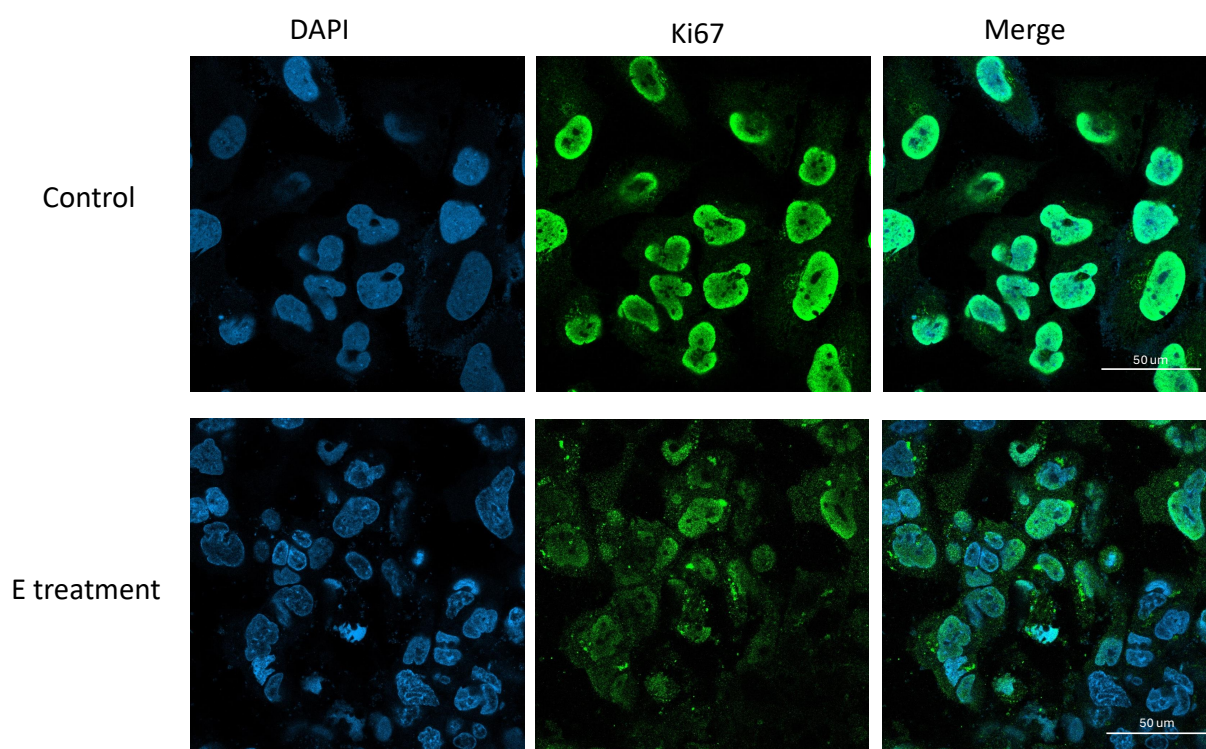


Figure 3.25 Ki-67 staining for proliferation analysis in astrocytes cells. Representative immunofluorescence images of U87 MG cells under control conditions (top row) and after exposure to the electric field (bottom row). DAPI (blue) stains cell nuclei, while Ki-67 (green) marks proliferating cells. The merged images (right column) show the colocalization of nuclei and proliferation marker expression. Scale bars: 20 μm .

In the control condition, the Ki-67 signal is high, as indicated by the intense green signal. Additionally, the cells appear healthy, with regular and homogeneous nuclei.

In contrast, the treated cells exhibit a completely different scenario. It is immediately evident that these cells are stressed, as their nuclei appear disrupted and highly irregular. The Ki-67 signal is significantly lower, and due to potential nuclear membrane damage, it is no longer confined to the nucleus but is instead dispersed in the cytoplasm. To further validate these observations, a quantification of the mean fluorescence intensity (MFI) was performed (see Figure 3.26)

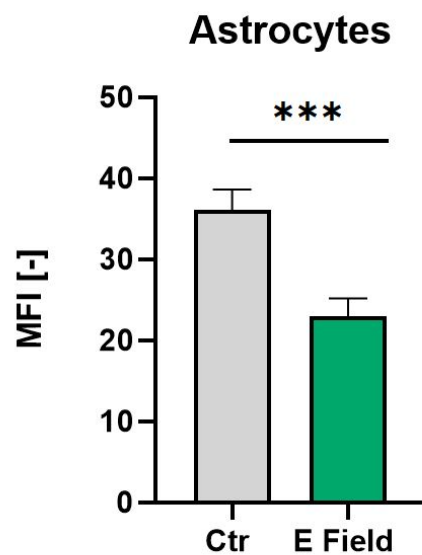


Figure 3.26 Column graph showing the Mean Fluorescence Intensity (MFI) of the Ki67 marker on astrocytes cells for quantification. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

Figure 3.25 presents a column graph showing the mean fluorescence intensity (MFI) calculated for control and exposed astrocyte cells. It is evident that the MFI of the control cells is significantly higher than that of the exposed cells, consistent with the confocal images (Figure 3.24).

Moreover, the ANOVA analysis reveals a statistically significant effect ($p < 0.001$), confirming that the decrease in MFI - and consequently, the reduced proliferation of astrocytes - is primarily attributed to exposure to the electric field.

3.2.5 pH variations analysis results

During the experiments, a change in the colour of the medium was observed after exposure, as shown in Figure 3.27 for both astrocytes and U-87 MG cells. Therefore, it is important to determine whether this change is significant and to investigate whether it is caused by the cells or by some component of the medium.

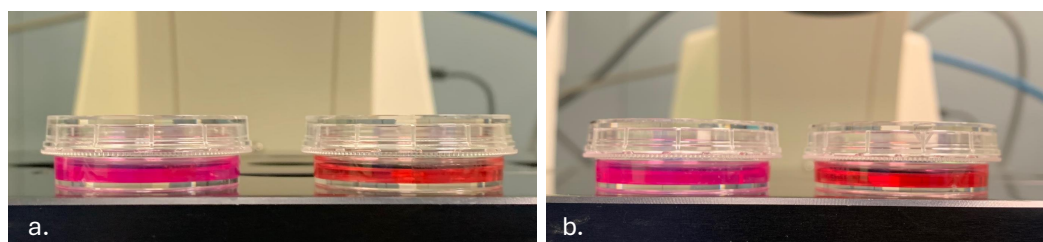


Figure 3.27 Changes in Medium Coloration After Exposure to the Electric Field. (a) Photograph of U-87 MG cell cultures: the Petri dish on the left was exposed to the electric field, while the one on the right served as the control. (b) Photograph of astrocyte cell cultures: the Petri dish on the left was exposed to the electric field, while the one on the right served as the control

To assess pH variations, measurements were taken for both the exposed medium, with and without cells, and compared to their respective control group, which were not exposed to the electric field. Statistical analysis compared the pH of the exposed and non-exposed medium without cells, but no significance differences were found. Since the variations observed in this condition were minimal, this comparison was excluded. Consequently, the statistical analysis focused only on the medium containing cells, comparing the exposed and non-exposed conditions.

3.2.5.1. U-87 MG cell line

The results of the statistical analysis for the pH variation of U-87 MG cells are presented in the column graph in Figure 3.28. The graph shows that, for U-87 MG cells, the pH value after exposure to the electric field is higher than that value of the control. This difference is statistically significant ($p < 0.0001$), as determined by the student t-test. Specifically, the pH of the medium containing cells increases after exposure, with a mean value of 9.3 compared to 8.15 in the control. After exposure, the cells cultures in the petri dish exhibited an increase in pH, leading to a shift towards a more basic condition. This shift caused the medium's color to change from red to purple, a phenomenon confirmed by Arora (2013).

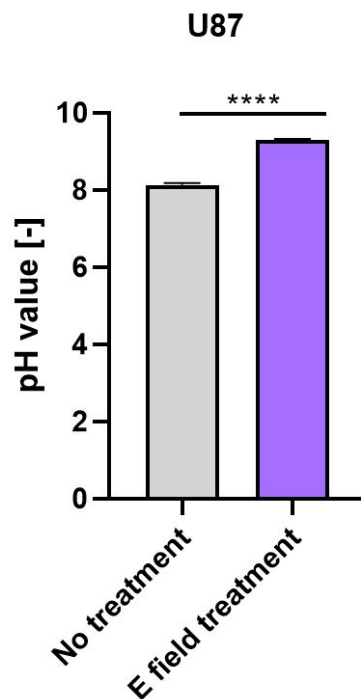


Figure 3.28 Column graph showing the result of the statistical analysis of the pH variation. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

Furthermore, as explained by Liberti & Locasale (2016) tumoral cells as U-87 MG are key player in the Warburg effect, which refers to their preference for glycolysis over cellular respiration, even in the presence of oxygen. This metabolic shift typically leads to increased lactate production, contributing to the acidification of the extracellular environment. However, in this case, an increase in pH was observed, indicating a shift toward a more basic condition. This is likely due to the fact that cell growth is strongly inhibited by the electric field, resulting in a reduction in the number of cells. Consequently, the medium is less consumed and contains lower lactate level, making it more basic compared to the control condition.

3.2.5.2 Astrocytes cell line

The results of the statistical analysis for the pH variation of astrocytes are reported in the column graph in Figure 3.29. Similar to U-87 MG cells, the statistical analysis shows that exposure to the electric field increases the pH of astrocyte cells inside the petri dish. The mean pH value is approximately 9, compared to 8.2 in the control.

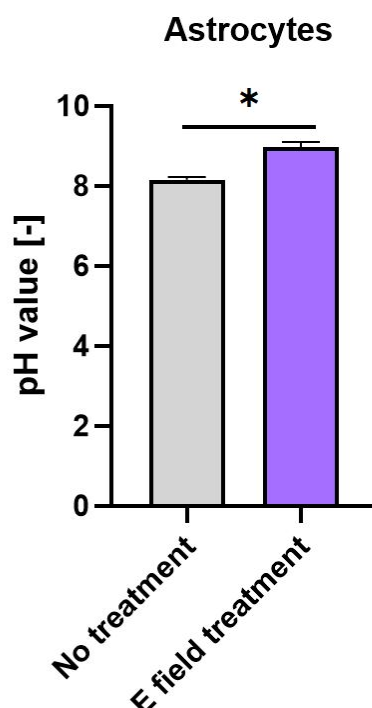


Figure 3.29 Column graph showing the result of the statistical analysis of the pH variation. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

This difference is statistically significant ($p < 0.05$) and was determined using Student t-test. The increase in the pH suggests that exposure to the electric field influences cellular metabolism, leading to a more basic environment, which in turn causes the color change from red to purple (Arora, 2013). This increase may be due to the inhibition of cell, resulting in a reduced number of cells in the medium. Consequently, the medium, is consumed less, making it more basic compared to the control condition.

3.2.6 Comparison of the electric field effect on the U-87 MG cells and astrocytes

In the previous sections, the results of each applied protocol were presented for both U-87 MG cells and astrocytes. One of the goals of this thesis was to understand and validate the differences in the effects of the electric field on the two cell lines. Therefore, a comparison between the results obtained for each is necessary and will now be presented. Analysis of the brightfield images indicates that the overall effect of the electric field on both U-87 MG cells (Figure 3.6) and astrocytes (Figure 3.10) appears very similar. After

exposure, both cell lines exhibit typical stress responses: they assume a rounder shape, their extensions retract, and many cells detach from the surface, leading to a general decrease in cell number. However, 24 hours after exposure, both cell lines show signs of recovery, attempting to regain their original morphology and increasing in number. After 48 hours, this recovery process becomes even more evident (Figure 3.7 and Figure 3.11). From a morphological perspective, Phalloidin and DAPI staining results (see section 3.2.2) confirm these observations. After exposure, both U-87 MG cells (Figure 3.14) and astrocytes (Figure 3.18) show fragmented F-actin filaments and a significant reduction in cell number. While recovery is observed at 24 and 48 hours, the F-actin filaments remain thinner, and although the cells attempt to regain their original morphology, their cytoskeletal organization remains disrupted. Notably, 48 hours after exposure, differences in cytoskeletal organization between the two cell lines become apparent. Due to the intrinsic polarized conformation of U-87 MG cells (Figure 3.16), their alignment appears less directional after exposure to the electric field. In contrast, for astrocytes (Figure 3.20), exposure leads to a more disorganized cytoskeleton, affecting their typical stellate morphology.

The MTT assay was performed to evaluate cell viability. Unexpectedly, U-87 MG cells exhibited higher viability than astrocytes immediately after exposure. The reason for this discrepancy is unclear but may be related to the timing of the test. Since the assay was conducted immediately after exposure, the cells were highly stressed, with many having detached, potentially affecting the results. It is possible that if the test had been performed after 24 hours later, the outcomes would have been different, with astrocytes displaying a higher survival rate.

In contrast to the MTT assay results, Ki-67 staining revealed that U-87 MG cells had a lower MFI value than astrocytes. This suggests that astrocytes may retain a slightly higher proliferative capacity under these conditions.

The pH variation analysed in Section 3.2.5 showed no significant differences between the two cell lines. Both environments exhibited an increase in pH, resulting in a more basic environment. This suggests that the electric field does not induce a cell-specific effect on pH regulation.

In conclusion, the electric field induced similar stress responses in both cell lines, causing cells to assume a rounder shape, detach, and lose cytoskeletal organization. However,

astrocytes appeared to struggle more in terms of viability immediately after exposure, whereas U-87 MG cells demonstrated a greater capacity to tolerate the initial stress. Over time, both cell lines attempted to recover, but F-actin organization remained altered, affecting their structural integrity. These findings suggest that while the electric field impacts both cell lines similarly, differences in their recovery dynamics and metabolic responses may be crucial in understanding their distinct adaptive mechanisms.

3.3 Biological validation: effects of the magnetic field

This section will analyze and discuss the biological effects induced by exposure to the magnetic field, following the same approach used for the electric field. First, morphological changes will be evaluated. Then, to assess cell viability and proliferation, the results from the MTT assay and Ki-67 staining will be examined. Finally, since a color change in the culture medium was observed during the experiments, pH measurements and analysis will be conducted.

3.3.1 Morphological changes in brightfield imaging

Brightfield microscopy images were analyzed to investigate the effects of magnetic field exposure on cell growth inhibition and early morphological changes, first in U-87 MG cells and then in astrocytes.

As previously explained in §2.1.2.1, the three petri dishes are placed inside the coil, stacked one on top of other. The applied frequency is 130 Hz, but the magnetic field varies depending on the position within the coil. The top dish experiences a 7 mT magnetic field, the middle one is exposed to 6 mT, and the bottom one to 5 mT. Different effects are expected based on the varying magnetic field strength to which the cells in the three specific positions are exposed. The exposure consists of a three-hour daily treatment for three consecutive days.

This section will first present the results of the first exposure, followed by those from the second and third. Finally, the results of the cells analyzed 24 hours after the last exposure will be examined to assess their ability to recover.

3.3.1.1. U-87 MG cell line

As with the electric field, a brightfield image of U-87 MG cells in the control condition is first shown in Figure 3.30. The cells appear well adhered to the surface, with a stellate conformation. Their branches are polarized and exhibit an organized arrangement (as also described in Figure 3.5).

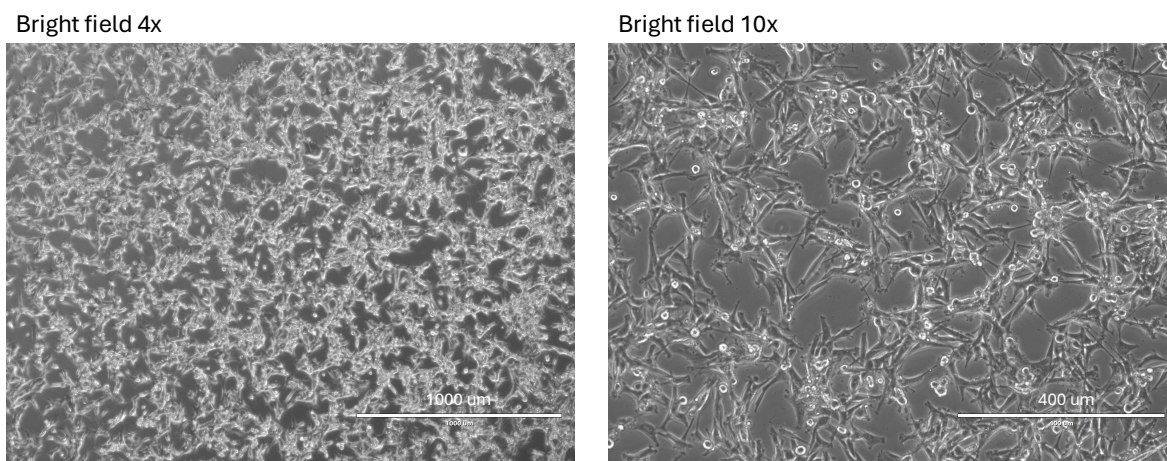


Figure 3.30 Brightfield microscopy images of U87 MG cells in the untreated control condition. (Left) Image at 4× magnification showing overall cell distribution. (Right) Image at 10× magnification displaying detailed cell morphology, with well-adhered cells exhibiting a stellate conformation and organized extensions.

In Figure 3.31 U-87 MG cells after the first magnetic field exposure are shown.

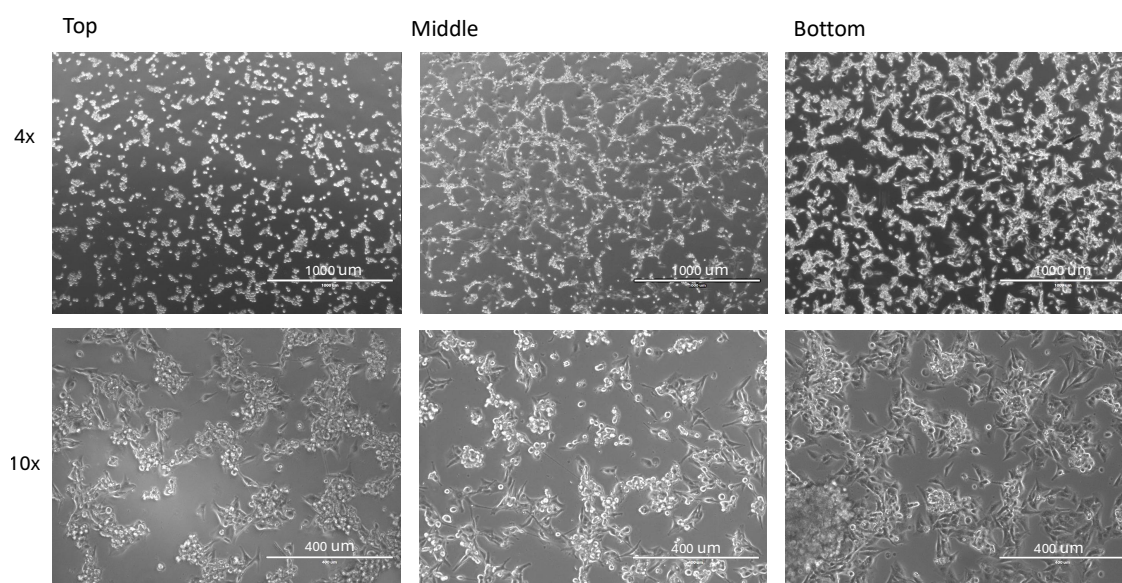


Figure 3.31 Brightfield microscopy images of U87 MG cells after the first exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

From a preliminary observation, it is evident that in all three petri dishes, after just one exposure, the cells appear rounder, and some detaching from the surface, leading to a

reduction in the number of attached cells. The cells in the petri dish positioned at the top of the coil are less elongated than those in the middle and bottom dishes. Additionally, they appear rounder and fewer in number. The cells in the middle petri dish are more numerous than those in the top and also exhibit a round shape; however, their original stellate morphology, which was observable in the control images (Figure 3.30), remains partially visible. Similarly, the cells in the bottom petri dish, which were exposed to the lowest magnetic field, appear more numerous and rounder, yet their original structure is still discernible.

Then, Figure 3.32 presents the effects on the cells after the second three-hours exposure.

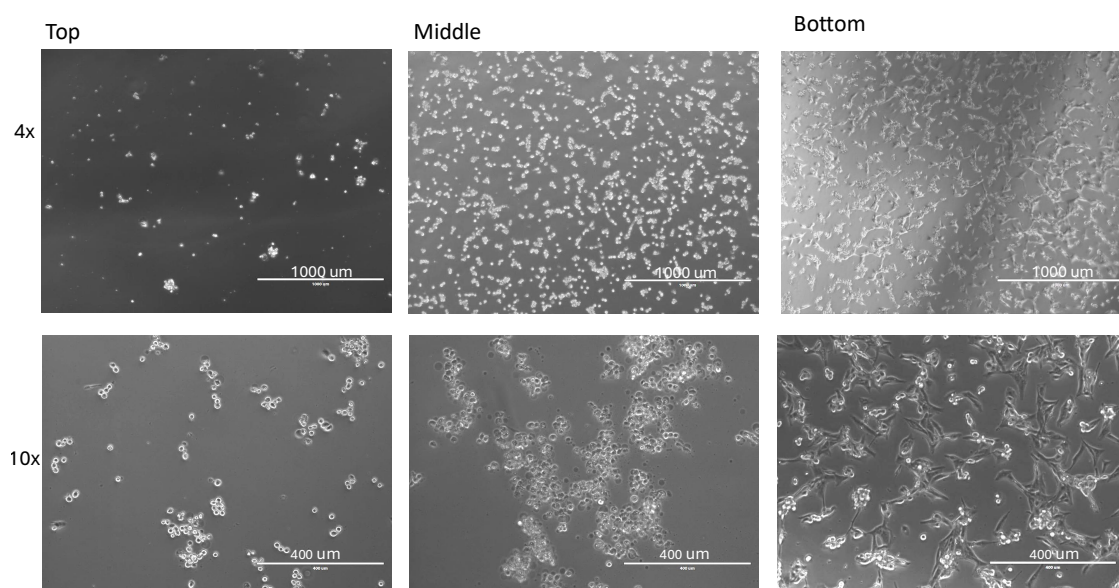


Figure 3.32 Brightfield microscopy images of U87 MG cells after the second exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

The effects of the magnetic field on the cells in the three different positions are more evident after the second exposure than those observed after the first exposure (Figure 3.31). Overall, the number of cells in all three petri dishes has decreased, with a particularly noticeable reduction in the top petri dish. Many of them have detached from the surface, appearing round and disorganized, with no trace of their original stellate morphology (Figure 3.30). In the middle petri, the cell count is slightly higher, but their round shape suggests imminent cell death.

The cells in the bottom petri dish still exhibit their original stellate shape, although they are less stretched than in the control. Some cells have detached from the surface and display a round shape as well.

In Figure 3.33, the results of the third magnetic field exposure are presented.

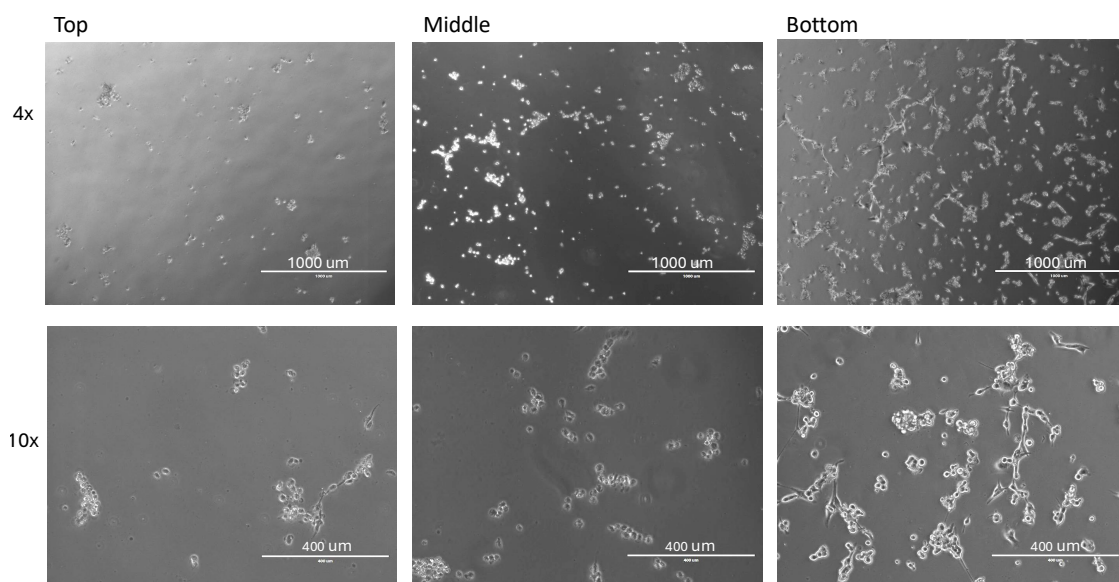


Figure 3.33 Brightfield microscopy images of U87 MG cells after the third exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After this exposure, most of the cells in the petri dish at the top of the coil are dead, with only a few remaining attached to the surface. However, their shape suggests significant damage, indicating a low probability of recovery.

The cells in the petri dish positioned in the middle of the coil also appear severely affected, exhibiting a round shape, with only a few still attached to the surface.

In contrast, the cells in the bottom petri dish are more numerous but display a disorganized morphology. The effects observed in the middle and top petri dishes are similar, whereas the cells at the bottom appear to be in relatively better condition overall.

Finally, Figure 3.34 presents the effects of the magnetic field on the cells after 24 hours after the last exposure.

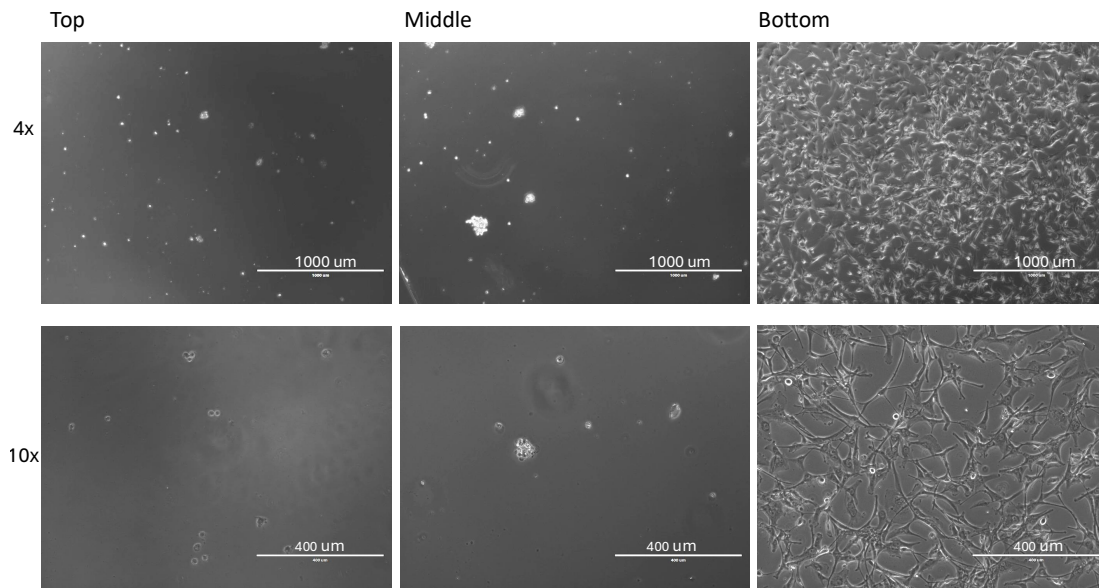


Figure 3.34 Brightfield microscopy images of U87 MG cells after 24 hours after the third exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After 24 hours, the cells in the top and middle petri dishes have shown no sign of recovery. Their number have further decreased, and almost all have detached from the surface, indicating cell death. In contrast, the cells in the petri dish at the bottom have recovered well, re-assumed their original stellate morphology, and significantly increased in number.

3.3.1.2 Astrocytes cell line

In Figure 3.35, images of untreated astrocytes in the control petri dish are shown. The cells appear well adhered to the surface, with a less polarized conformation compared to the U-87 MG cells but still well organized, as previously highlighted in §3.2.1.2 (Figure 3.9).

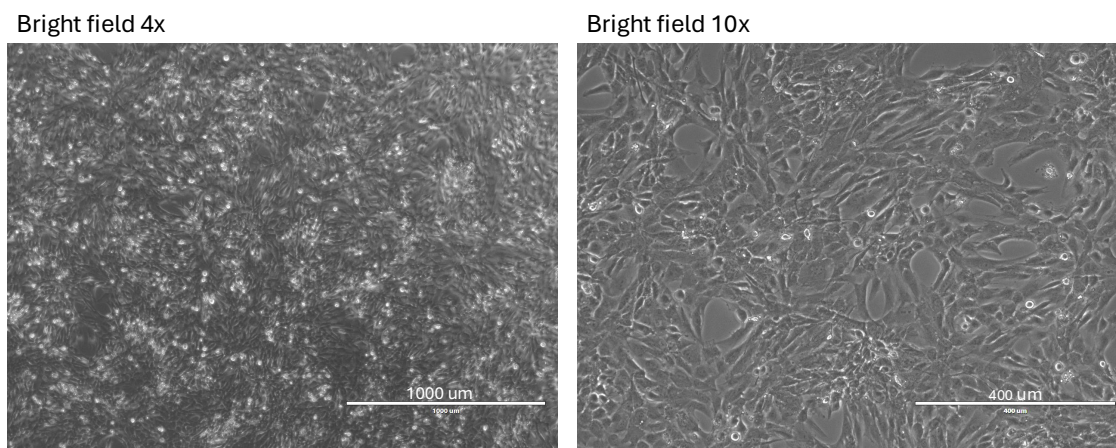


Figure 3.35 Brightfield microscopy images of Astrocyte cells in the untreated control condition. (Left) Image at 4× magnification showing overall cell distribution. (Right) Image at 10× magnification displaying detailed cell morphology, with well-adhered cells exhibiting organized extensions.

In Figure 3.36 the results of the first magnetic field exposure are shown.

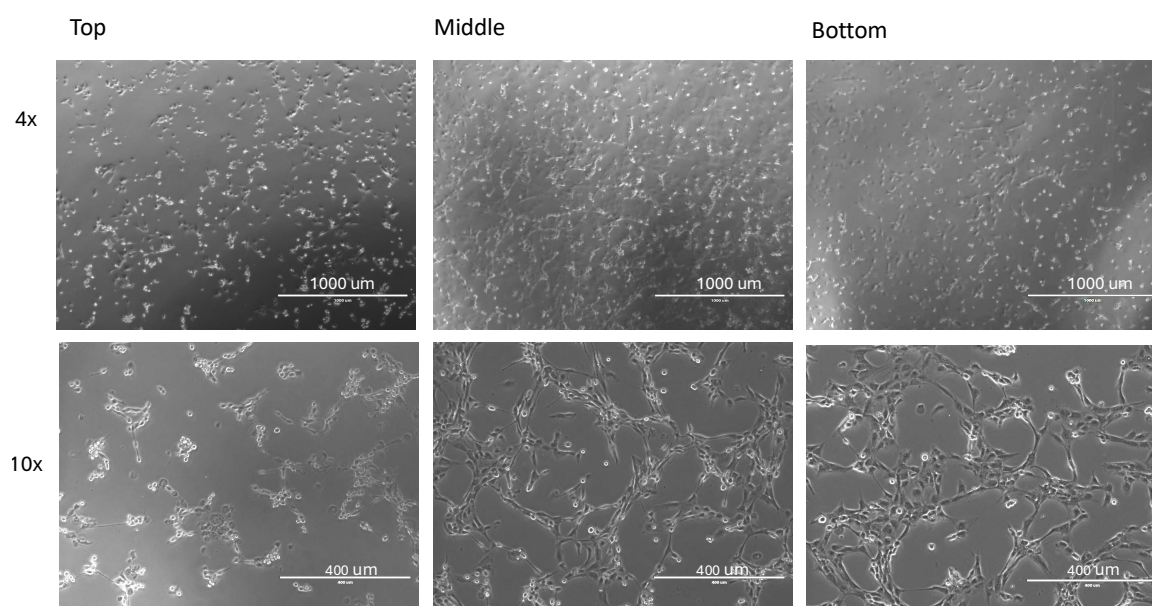


Figure 3.36 Brightfield microscopy images of Astrocyte cells after the first exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

The effects of the field on the three different petri dishes appear slightly different. In the petri dish at the top of the coil, the number of cells is lower due to some cells detaching from the surface. Their shape is rounder and less stretched compared to the control (Figure 3.35). In the middle and bottom petri dishes, the number of cells remains high, but at 10x

magnification, some small differences can be observed: the cells in the middle petri dish are rounder and thinner, while those in the bottom petri dish appear well spread.

Figure 3.37 shows the cells after the second exposure to the magnetic field.

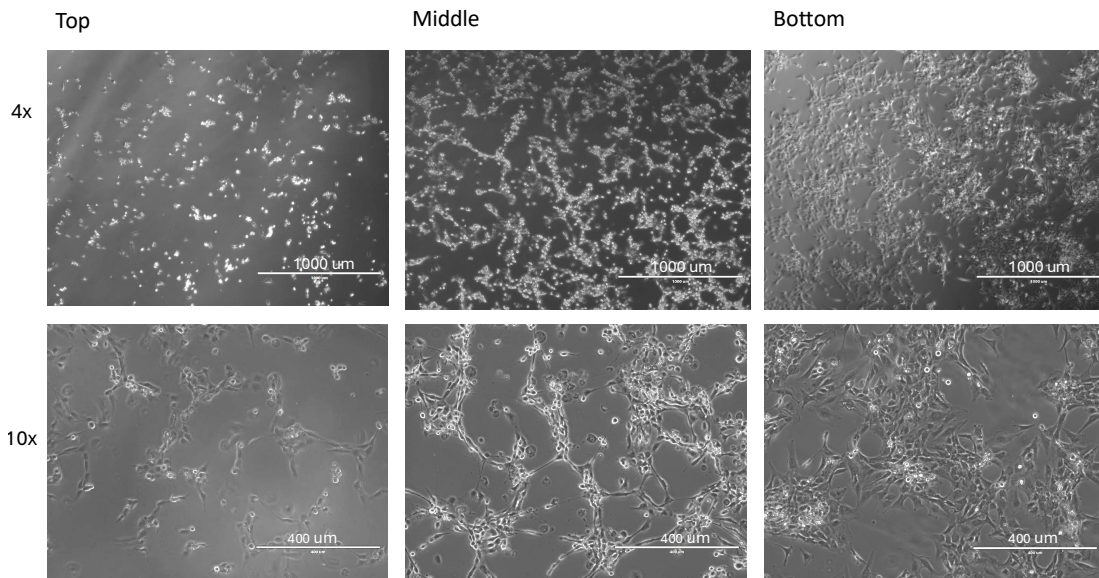


Figure 3.37 Brightfield microscopy images of Astrocyte cells after the second exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After the second magnetic field exposure, the effects on the three petri dishes differ. In the petri dish at the top, the majority of the cells have adopted a rounded morphology, although some have managed to maintain slight spreading with branches. Additionally, many cells have detached from the surface. The cells in the petri dish in the middle have somewhat preserved their elongated conformation, though some have detached and assumed a rounded shape as well. In the petri at the bottom, the cells appear well-adhered to the surface, and their conformation is similar to that of the control. Their number has not decreased, indicating that during the time they were not exposed, they have grown.

Figure 3.38 shows the cells after the third magnetic field exposure.

After the third exposure, the effects of the magnetic field on the three petri dishes are strongly different compared to what was observed after the first and second exposure. The cells at the top are predominantly dead, exhibit a round shape, and are detached from the surface, suggesting that they will not recover. Their number have severely decreased compared to how they were after the second exposure (Figure 3.37). The cells in the petri dish in the middle seem in better condition, have remained attached to the surface, and are still rounder than in the control (Figure 3.35), but some stretching is still observed. The cells in the bottom petri are rounder due to the stress from the exposure, but at 10x magnification, it can be seen that they are still stretched, indicating a possible recovery.

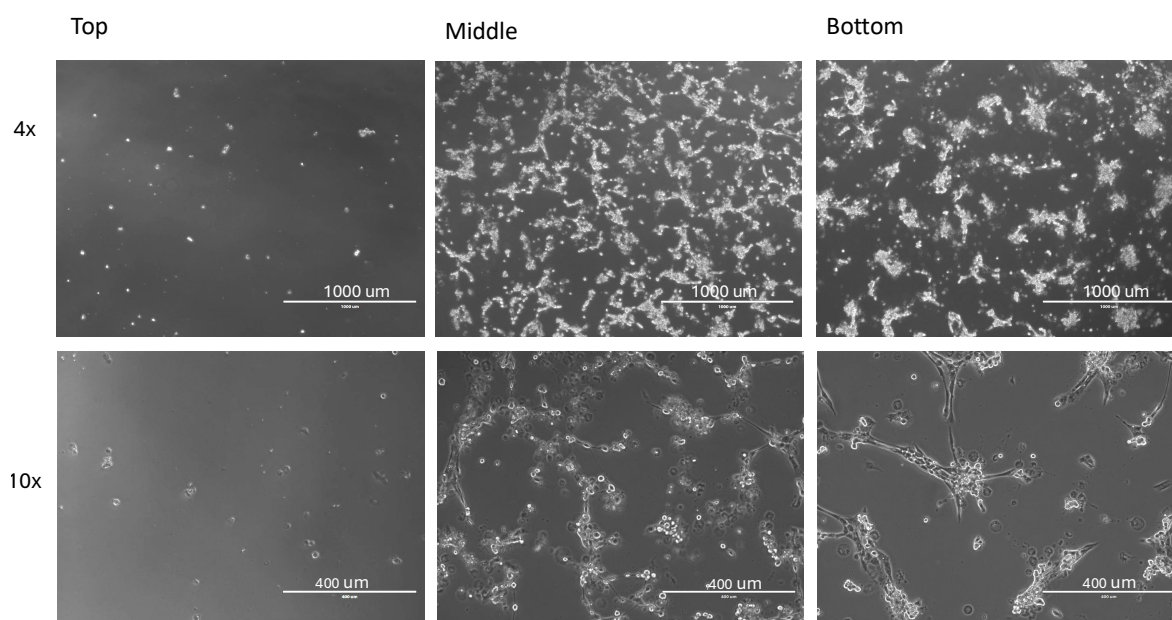


Figure 3.38 Brightfield microscopy images of Astrocyte cells after the third exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

Finally, Figure 3.39 presents the cells 24 hours after the last magnetic field exposure.

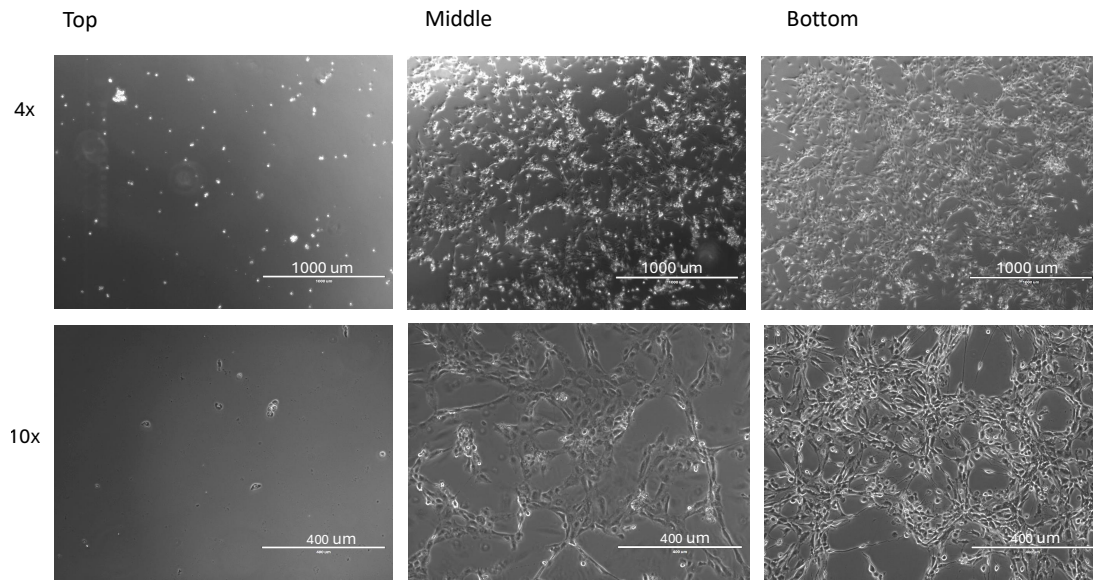


Figure 3.39 Brightfield microscopy images of Astrocyte cells 24 hours after the third exposure to the magnetic field. Images are taken at 4× (top row) and 10× (bottom row) magnifications for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

As expected, the cells in the petri at the top have not recovered, and their condition is consistent with what was observed in Figure 3.38. The cells in the middle and bottom petri dishes appear to have recovered, their number has increased, and their conformation looks very similar to that of the control (Figure 3.35). In both petri dishes, the cells appear stretched and well attached to the surface. In general, after exposure, astrocytes fail to re-assume their interconnected network, which is crucial for maintaining neural homeostasis and support.

3.3.2 Morphology changes observed with phalloidin and DAPI staining

In this section, the results of phalloidin and DAPI staining following magnetic field exposure are reported. The time points analyzed remain the same as before for U-87 MG cells: control condition, immediately after the first, second, and third exposure, and 24 hours after the last exposure. For astrocytes, however, only three time points will be considered: control condition, after all three exposures, and 24 hours after the last exposure. For tumor cells, a detailed analysis was necessary after each exposure to

assess its specific effects. In contrast, for healthy cells, the focus is on evaluating the overall impact of magnetic field exposure to determine whether the damage is reversible

3.3.2.1. U-87 MG cell line

Figure 3.40 shows the results of the phalloidin and DAPI staining for the U87 MG cells

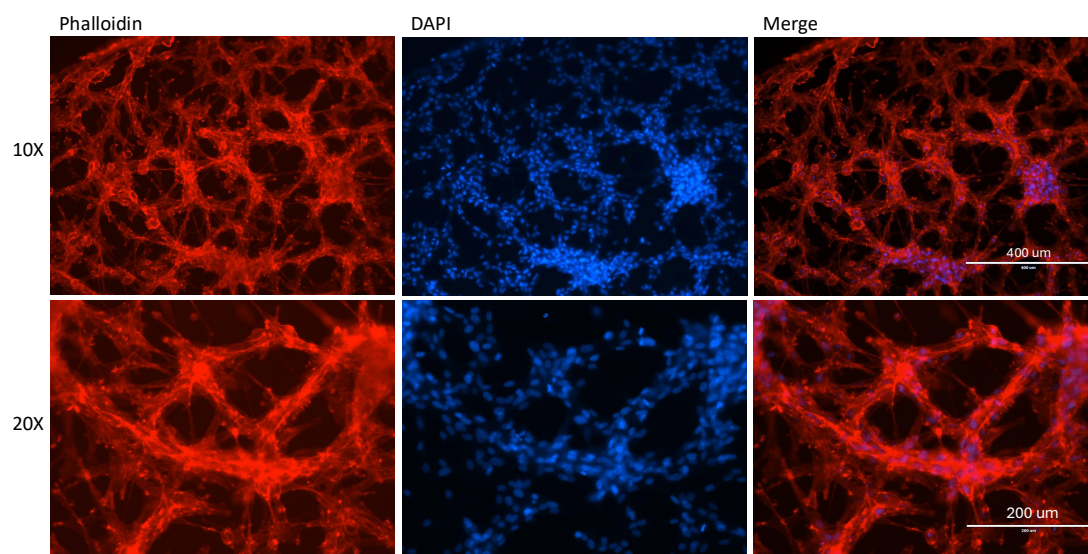


Figure 3.40 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells before treatment, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10×) and 200 μm (20×).

As seen in the brightfield images, the cells display an elongated morphology, characterized by F-actin extensions that are essential components of the cytoskeleton. The cytoskeleton plays a crucial role in maintaining cellular structure and shape. The nuclei, stained blue, are abundant and properly positioned in relation to the F-actin network. The high proliferation rate is reflected by the large number of nuclei and the well-developed filamentous actin.

Figure 3.41 and Figure 3.42 show the results of the phalloidin and DAPI staining for the cells after the first magnetic field exposure at 10x and 20x magnification, respectively

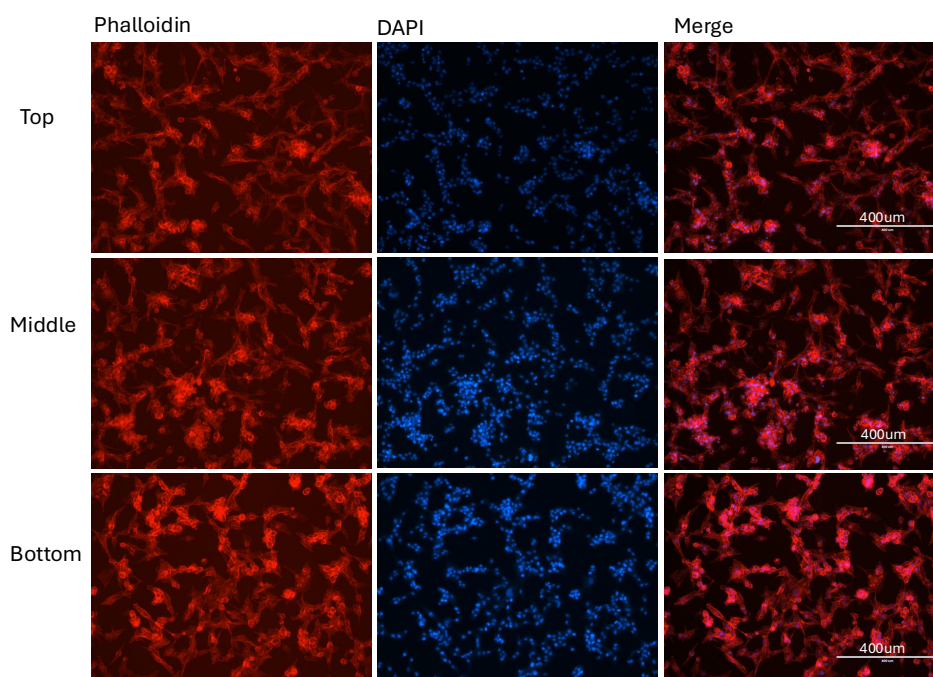


Figure 3.41 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after the first magnetic field exposure, imaged at 10 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μ m (10 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

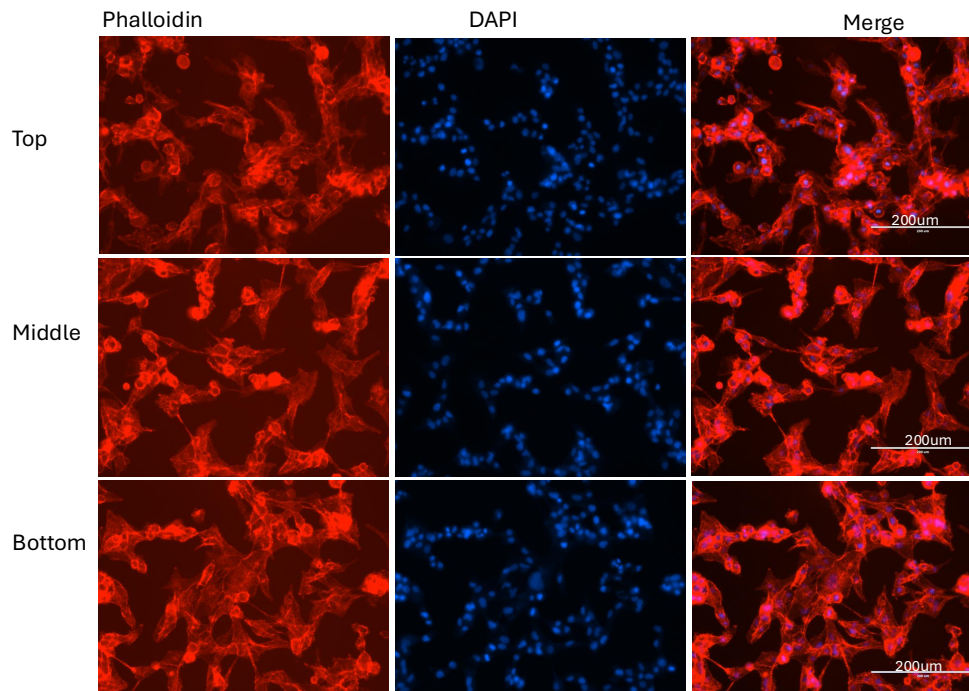


Figure 3.42 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after the first magnetic field exposure, imaged at 20 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 200 μ m (20 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After the first exposure, it can be seen that the morphological stellate organization of the U-87 MG cells is completely lost. The F-actin extension look fragmented in all the three petri dishes, and there are no visible differences between the three petri at the three different positions.

Figure 3.43 and Figure 3.44 show the results of the phalloidin and DAPI staining after the second magnetic field exposure at 10x and 20x magnification, respectively.

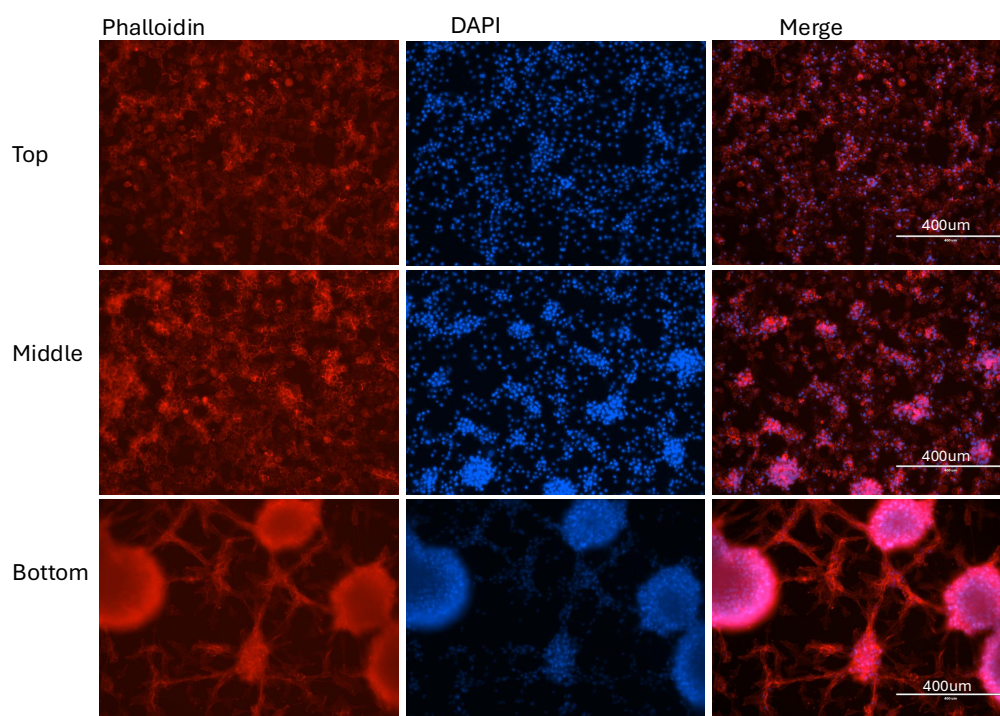


Figure 3.43 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after the second magnetic field exposure, imaged at 10× magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 µm (10×) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

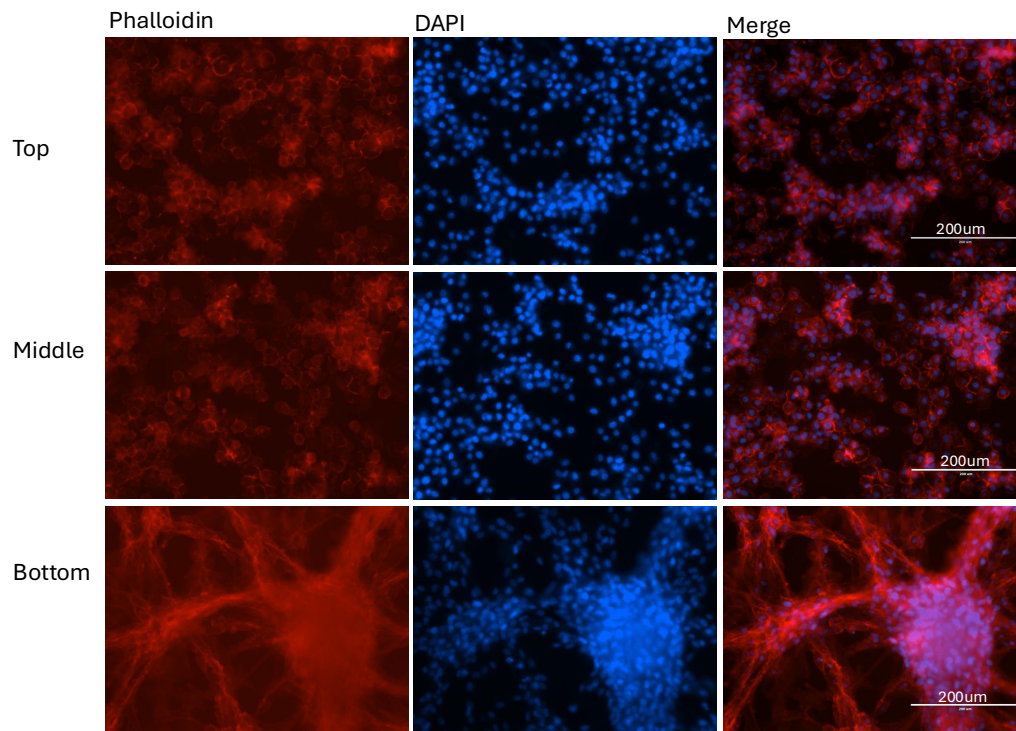


Figure 3.44 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after the second magnetic field exposure, imaged at 20 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 200 μ m (20 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After the second exposure, the effects in the three petri dishes become more evident, especially between the top and middle petri dishes compared to the bottom one. In the top and middle petri dishes, the F-actin filaments remain fragmented, similar to what was observed after the first exposure (Figure 3.41 and Figure 3.42). The fragmentation of the F-actin extension and so the modification of the cytoskeleton after exposure are results also supported by Teodori et al. (2006). The nuclei appear disorganized, but they are still well arranged in respect of the F-actin filaments. The cells in the bottom petri dish appear adhered and, during the period in which they were not exposed, managed to reorganize into clusters. After exposure, they do not seem particularly stressed, but the filaments connecting the various clusters are thinner than those in the control (Figure 3.40). This suggests that the magnetic field had an effect on the cellular network, although it was not strong enough to disrupt the clusters.

This phenomenon could be explained by the influence of magnetic fields on cellular structures involved in ion transport and signaling. Some studies suggest that magnetic fields may alter the organization or dynamics of the cytoskeleton, potentially affecting cell connectivity and communication (Gartzke & Lange, 2002). The observed thinning of the connecting filaments might reflect subtle modifications in cytoskeletal remodeling, without compromising overall cell adhesion or cluster integrity.

Furthermore, Figure 3.45 and Figure 3.46 show the results of the phalloidin and DAPI staining for the cells after the third magnetic field exposure at 10x and 20x magnification, respectively

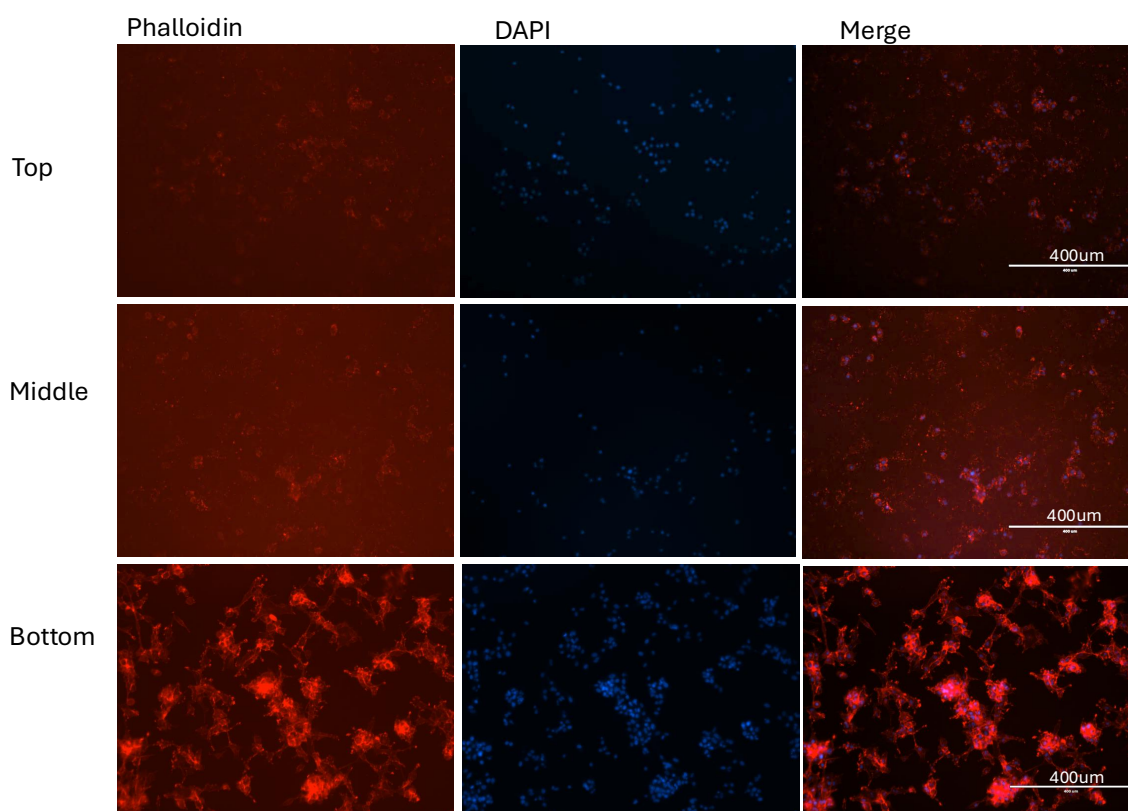


Figure 3.45 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after the third magnetic field exposure, imaged at 10 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μ m (10 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

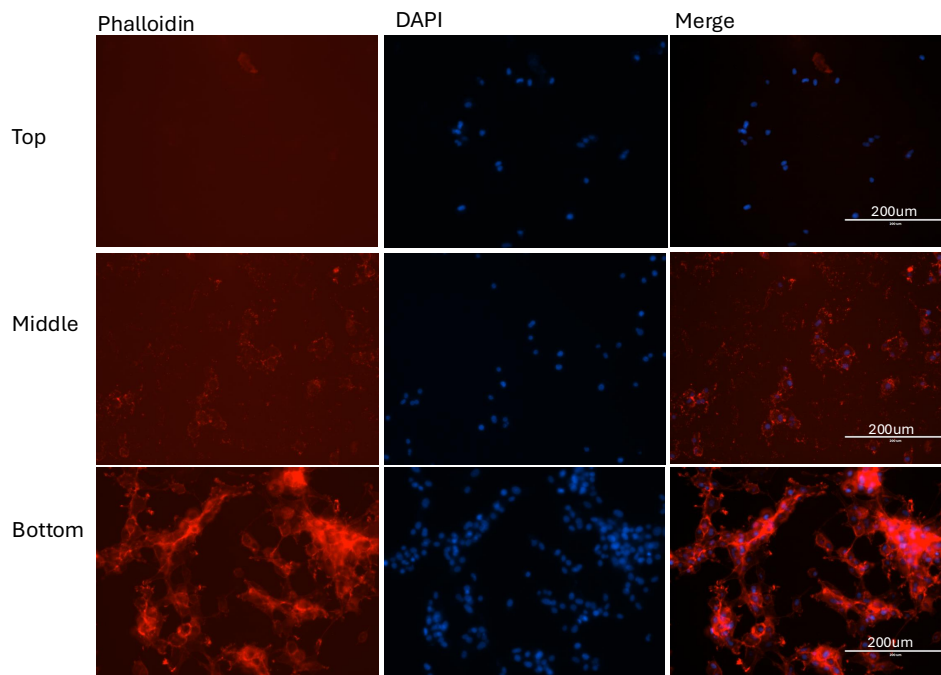


Figure 3.46 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells immediately after the third magnetic field exposure, imaged at 20 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 200 μm (20 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After the third exposure, it is evident that the cells in the top and middle petri dishes are in a severely compromised state. The number of the cells is significantly decreased, as observed in the brightfield images, with F-actin extensions absent and only a few nuclei remaining.

The situation is different for the cells in the bottom petri dish. While they appear stressed, their F-actin filaments are fragmented but still present. The clusters observed after the second exposure (Figure 3.44) are no longer visible. The cells are highly disorganized, and it appears their number has decreased.

Finally, Figure 3.47 presents the results of the phalloidin and DAPI staining on the cells 24 hours after the last exposure. The image shows only the cells in the bottom petri dish, as the cells in the top and middle petri were in the same condition as after the third

exposure: most of the cells are dead, and the microscope did not detect any signal due to their complete death.

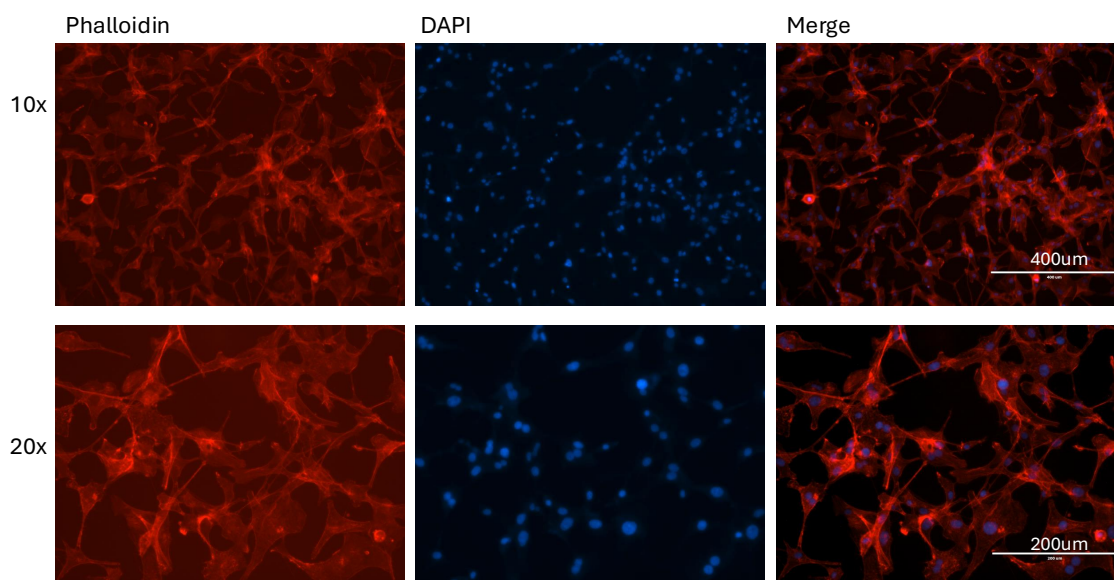


Figure 3.47 Results for U-87 MG staining. Phalloidin (red) and DAPI (blue) staining of U87 cells after 24 hours after the third magnetic field exposure, imaged at 10× (top row) and 20× (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 µm (10×) and 200 µm (20×). Only the petri in the **Bottom** (5mT)

After 24 hours, the cells in the bottom petri dish seem to be starting to recover from the exposure. However, the thin and disorganized F-actin filaments clearly show that the cells are unable to regain their original conformation. The number of nuclei has increased, suggesting that they may recover if they are not subjected to further stress.

3.3.2.2 Astrocytes cell line

Firstly, Figure 3.48 shows the results of the phalloidin and DAPI staining in the control condition for the astrocytes.

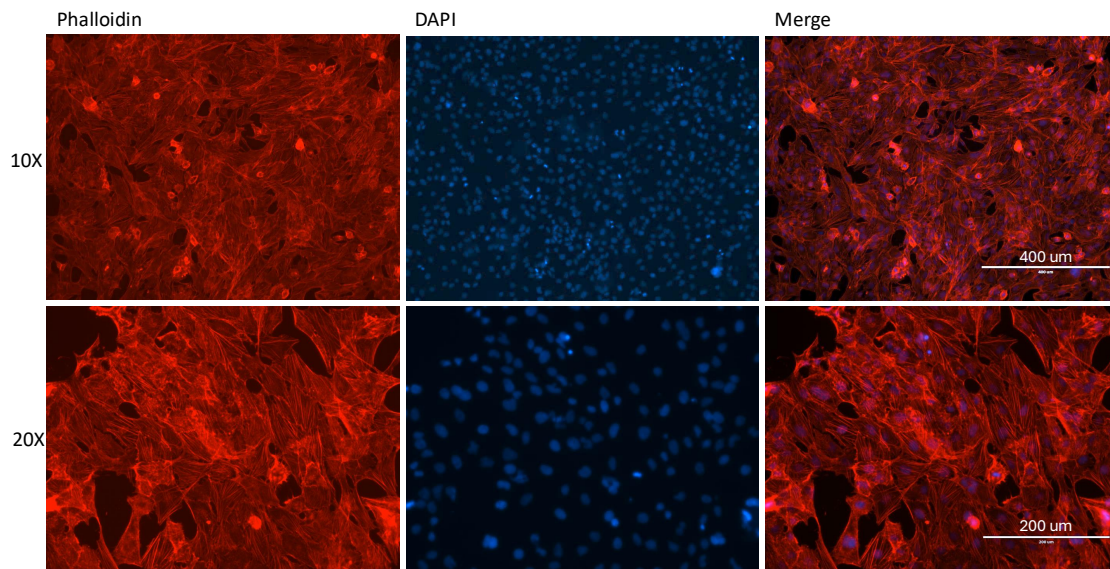


Figure 3.48 Results for Astrocyte cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocyte before treatment, imaged at 10 $\times$ (top row) and 20 $\times$ (bottom row) magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μm (10 $\times$) and 200 μm (20 $\times$).

The astrocytes in the control condition are well-organized, with evenly distributed nuclei. The cells are compact, forming a dense network with well-structured F-actin filaments. Figure 3.49 and Figure 3.50 show the result of the phalloidin and DAPI staining for the astrocytes cells after three magnetic field expositions at 10x and 20x magnification, respectively.

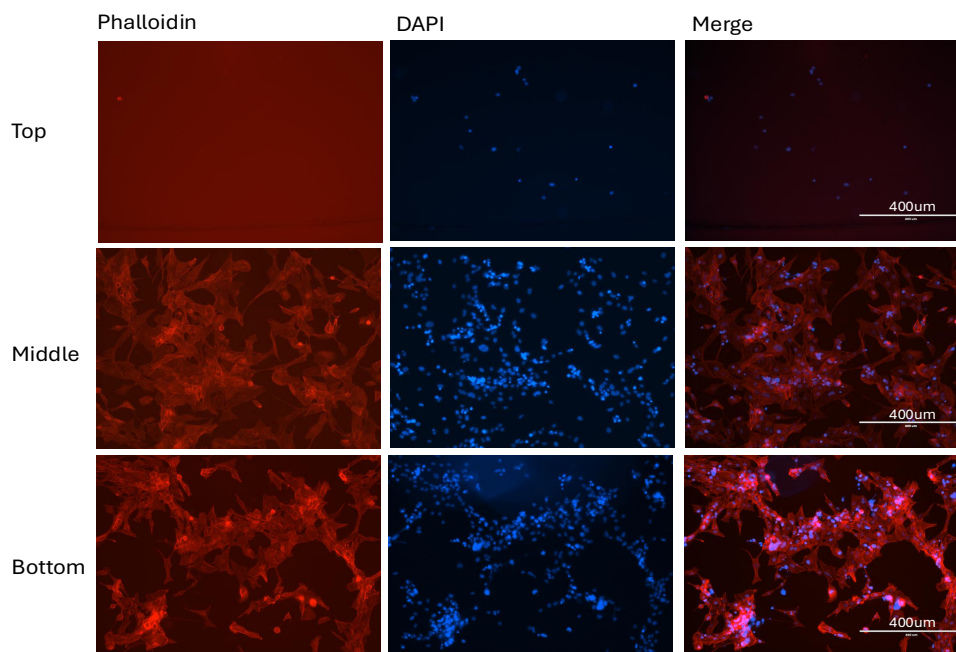


Figure 3.49 Results for Astrocytes cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocytes cells immediately after the third magnetic field exposure, imaged at 10 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μ m (10 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

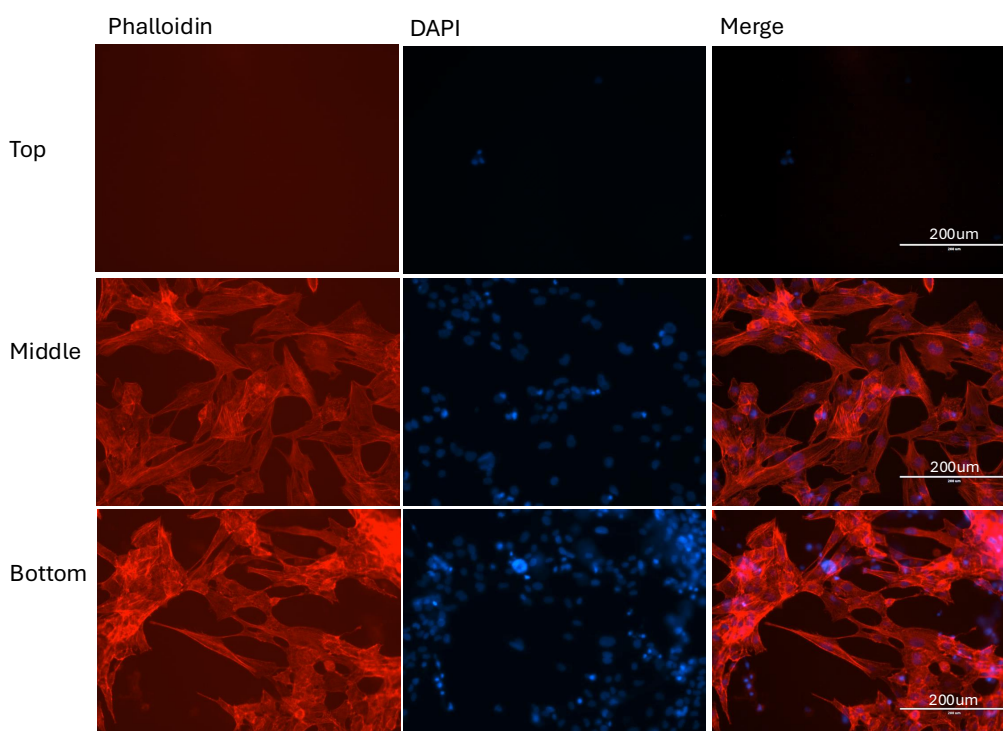


Figure 3.50 Results for Astrocytes cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocytes cells immediately after the third magnetic field exposure, imaged at 20 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 200 μ m (20 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

It is evident that the cells are in a completely different state compared to the control. In the top petri dish, the cells are completely dead, the F-actin extensions are absent, and only a few nuclei can be observed.

In the middle petri dish, the cells are noticeably more numerous, and the F-actin filaments are visible. However, compared to the control, they appear more disorganized, thinner, and less compact. A similar situation is observed in the bottom petri dish.

Finally, Figures 3.51 and 3.52 show the staining results of the astrocytes after 24 hours after the third exposure at 10x and 20x magnifications, respectively.

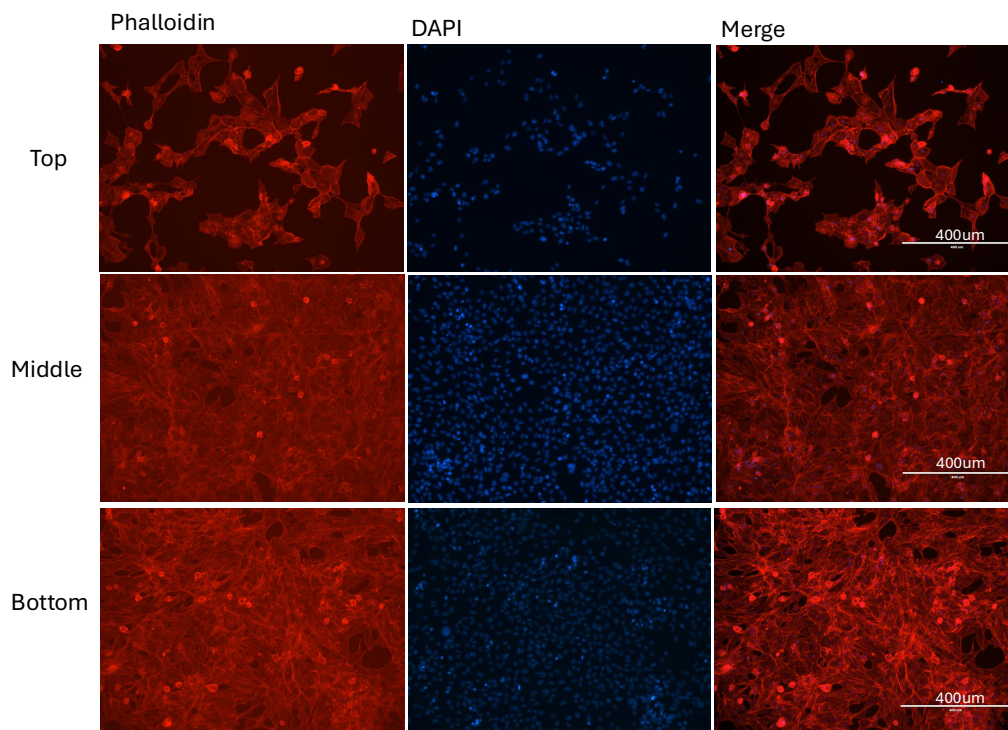


Figure 3.51 Results for Astrocytes cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocytes cells after 24 hours from the third magnetic field exposure, imaged at 10 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 400 μ m (10 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

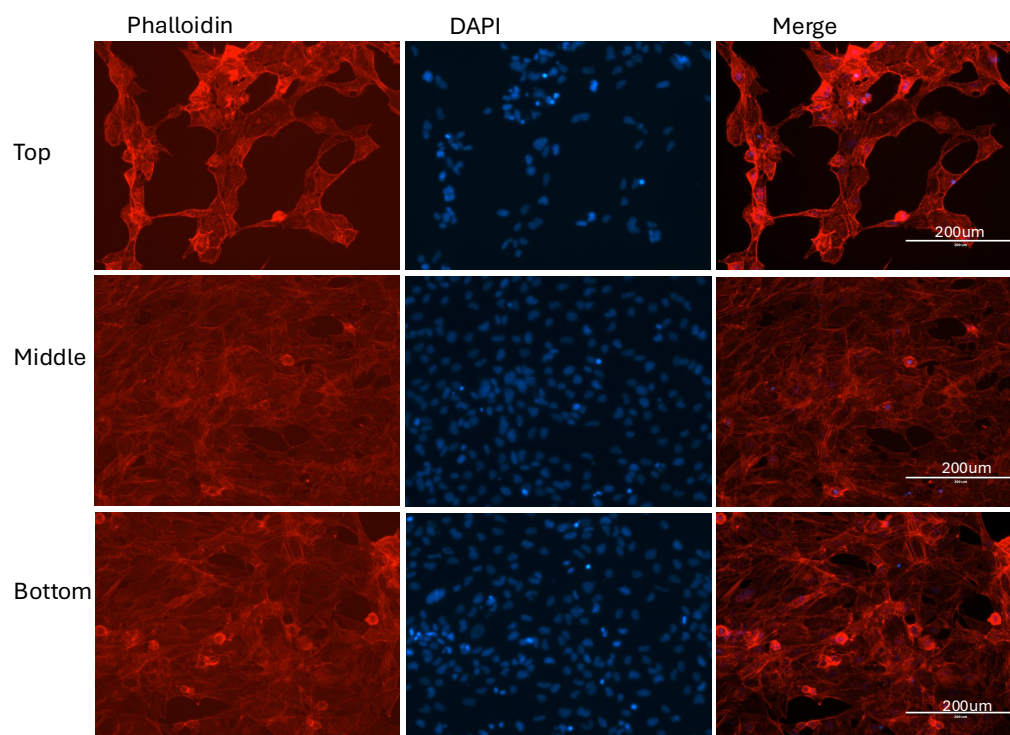


Figure 3.52 Results for Astrocytes cells staining. Phalloidin (red) and DAPI (blue) staining of Astrocytes cells immediately after 24 hours from the third magnetic field exposure, imaged at 20 $\times$ magnification. The merged images (right column) show the cytoskeletal structure and nuclear staining. Scale bars: 200 μ m (20 $\times$) for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

After 24 hours from the last exposure, the cells in the top petri dish appear to be recovering from the stress they were exposed to. Their morphology is very different from the control but improved compared to their appearance immediately after the last exposure (Figures 3.49 and 3.50), indicating that cells which had detached from the surface may have reattached during the 24 hours they were not exposed. The cells in the middle and bottom petri dishes appear to have mostly recovered; they have resumed their branched morphology, although the F-actin filaments look more compact than in Figures 3.49 and 3.50, but still thinner than in the control (Figure 3.48).

3.3.3 MTT assay results

In order to investigate the cellular viability following the magnetic field exposures, the MTT viability assay will be performed. The protocol was applied to the two different cell types immediately after the last exposure to the magnetic field.

The results of the MTT assay, presented as a column graph, quantify the viability of U-87 MG cells and astrocytes after magnetic field exposure. Statistical analysis was performed using a Student t-test to compare treated and control groups for each petri dish in its specific position. To assess the statistical differences between all three petri dishes in different positions compared to the control, an ANOVA test was used.

3.3.3.1. U-87 MG cell line

The results of the MTT assay for the U-87 MG cells are reported in the graphs in Figure 3.53.

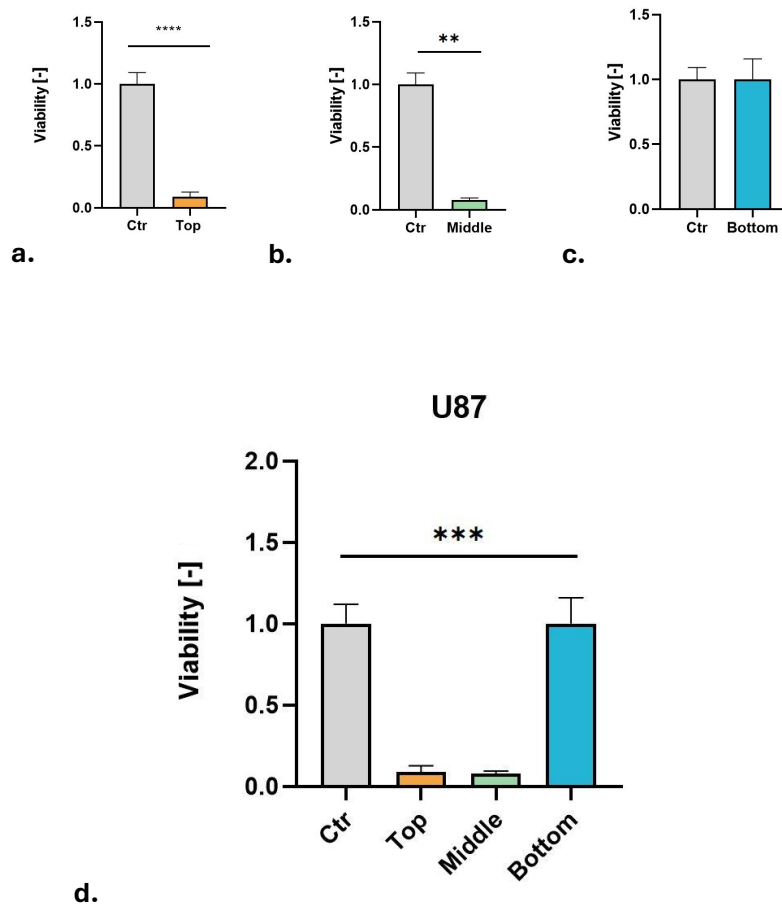


Figure 3.53 Results for the MTT assay for the U-87 MG cells immediately after the third magnetic field exposure. (a) Column graph of the MTT assay performed on the cells inside the top petri. (b) Column graph of the MTT assay performed on the cells inside the middle petri. (c) Column graph of the MTT assay performed on the cells inside the bottom petri (d) Column graph of the MTT assay with all the different petri compared with the control All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

The MTT assay evaluates cell viability. In particular, for the cells in the top petri (Figure 3.53a), statistical analysis indicates a highly significant reduction in cell viability compared to the control ($p < 0.0001$). This result is also strongly consistent with the brightfield images (Figure 3.33) and the phalloidin and DAPI staining (Figure 3.46), which clearly show that the cells were in poor condition.

Similarly, for the middle petri, Figure 3.53(b) shows a notably significant decrease in cell viability ($p < 0.01$) compared to the control, which is remarkably similar to the result observed in the top petri dish. Moreover, this outcome is also closely aligned with the observations from the brightfield images and staining images results.

In contrast, in the bottom petri, Figure 3.53(c) indicates no statistically significant difference, with cell viability essentially comparable to the control. Once again, this finding is also strongly supported by the brightfield and staining images.

Finally, Figure 3.53d presents the overall statistical analysis of the three samples relative to the control, confirming a statistically significant difference ($p < 0.001$) among the different groups.

Overall, the results obtained are perfectly consistent with the fact that a higher magnetic field clearly corresponds to stronger inhibitory effects, while a lower magnetic field results in weaker effects.

3.3.3.2 *Astrocytes cell line*

The results of the MTT assay for the astrocytes are presented in the following column graphs. All the statistical analyses performed in the four cases were not statistically significant. Regarding the top (Figure 3.54a) and middle (Figure 3.54b) petri dishes, it can be observed that cell viability is significantly lower than that of the control, as expected from the analysis of the brightfield images and the results of the phalloidin and DAPI staining. In contrast, in the bottom petri, cell viability is similar to that of the control, which is also consistent with the bright-field images (Figure 3.37) and the phalloidin and DAPI staining results (Figure 3.50)

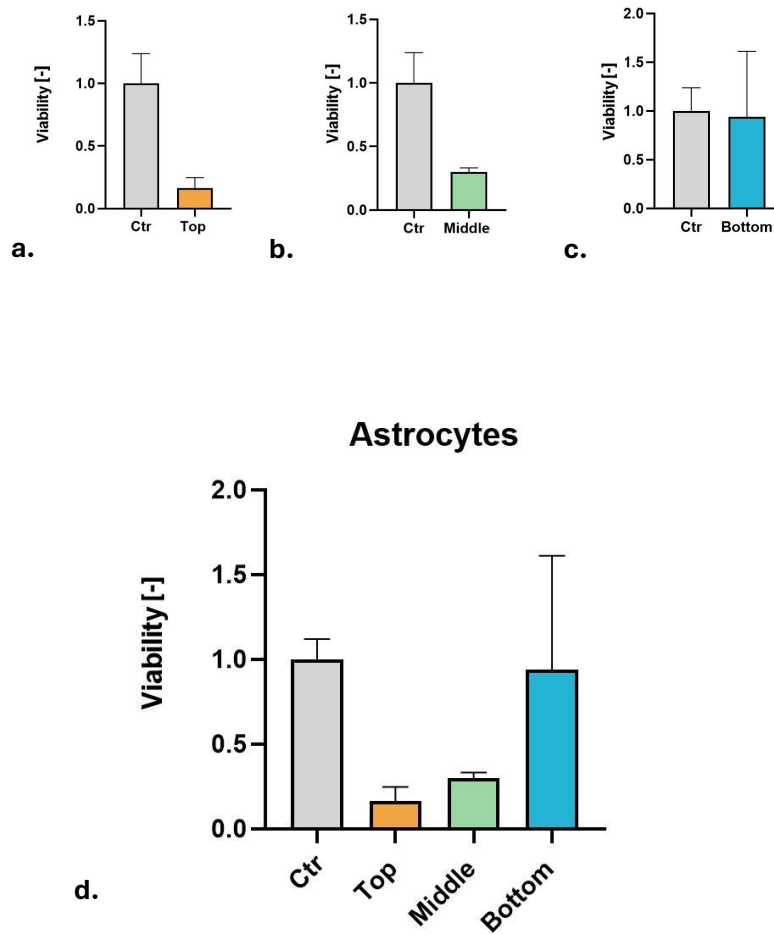


Figure 3.54 Statistical analyses of the MTT results for the astrocytes (a) Column graph of the MTT assay performed on the cells inside the top petri. (b) Column graph of the MTT assay performed on the cells inside the middle petri. (c) Column graph of the MTT assay performed on the cells inside the bottom petri (d) Column graph of the MTT assay performed on all the different petri compared with the control.

All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

3.3.4 Immunofluorescence with marker Ki-67: results

As done for the electric field in §3.2.4, the ability of the cells to proliferate after exposure to the magnetic field is investigated through Ki-67, a proliferation marker. This is done by comparing the results of the exposed samples with those of the control. The fluorophore used for Ki-67 is green, meaning that the greater the green intensity, the higher the proliferative capacity of the cells.

Initially, the results for the U-87 MG cells will be presented, followed by the results for astrocytes exposed to the magnetic field.

3.3.4.1. U-87 MG cell line

In Figure 3.55, the images of the cells stained with Ki-67 for the control condition and the middle and bottom petri dishes are reported. The top petri dish was excluded because, after exposure, all the cells detached from the coverslip, preventing them from being stained with Ki-67.

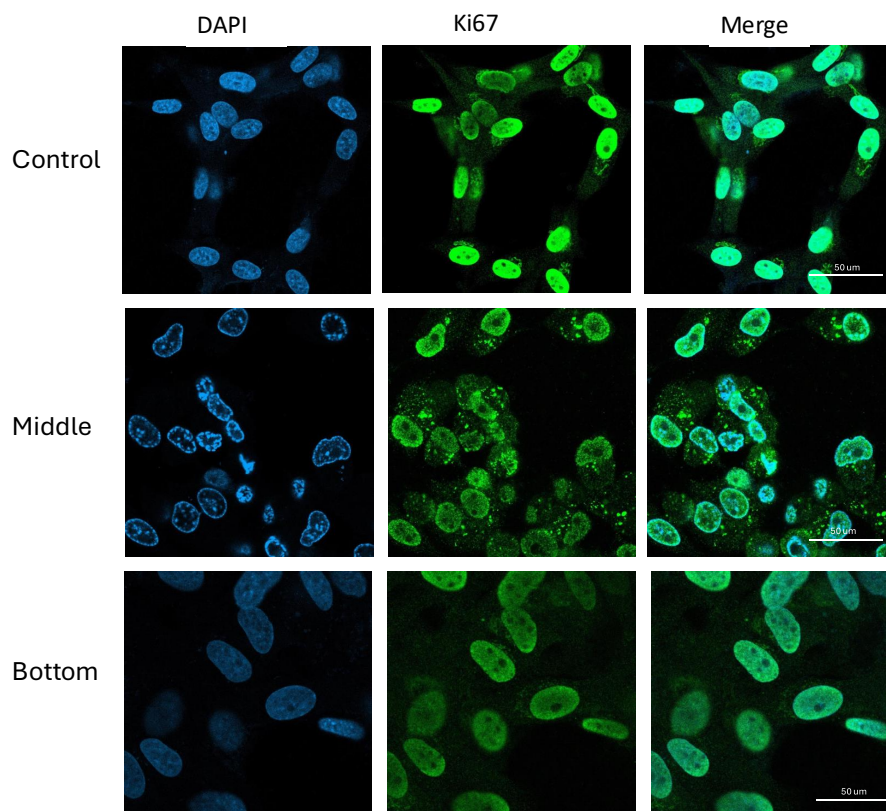


Figure 3.55 Ki-67 staining for proliferation analysis in U-87 MG cells. Representative immunofluorescence images of U87 MG cells under control conditions (top row) and after the third exposure to the magnetic field (bottom row). DAPI (blue) stains cell nuclei, while Ki-67 (green) marks proliferating cells. The merged images (right column) show the colocalization of nuclei and proliferation marker expression. Scale bars: 20 μm for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

The nuclei of the cells in the control condition have a regular shape and a distribution consistent with what was observed in Figure 3.40. The Ki-67 marker is strongly evident, indicating a significant proliferative capacity.

In the middle petri dish, the situation changes: the shape of the nuclei is severely altered, irregular, and varies in size. Despite the inhibitory effects of the magnetic field observed in the previously sections, Ki-67 remains clearly visible.

For the bottom petri dish, as expected, the situation is more similar to the control. The nuclei maintain a regular shape, and the Ki-67 marker is less pronounced than in the control due to exposure to the magnetic field. However, the marker remains well confined within the nuclei, indicating their integrity.

To statistically confirm the observations made from the images (Figure 3.55), a quantitative analysis of fluorescence intensity was performed to precisely quantify the proliferative capacity of the cells, using the mean fluorescence intensity (MFI) as an indicator.

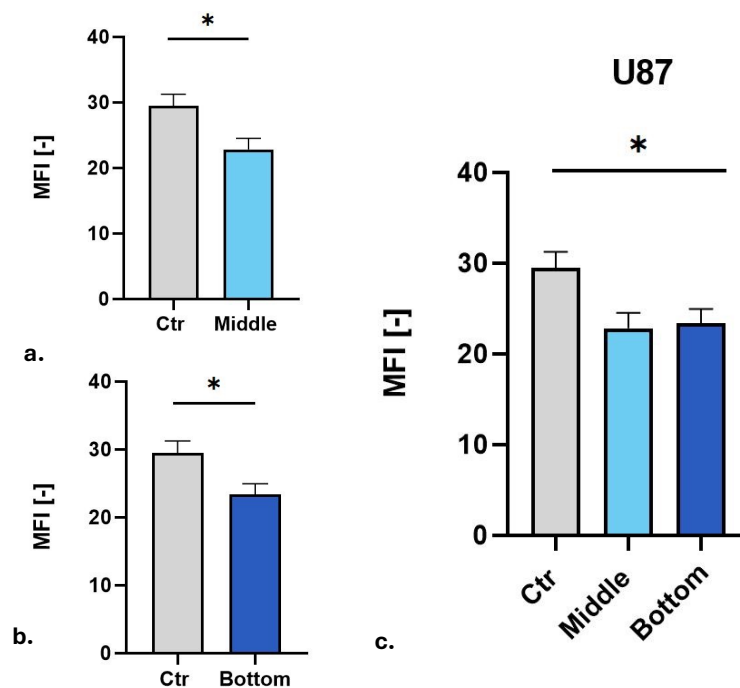


Figure 3.56 Column graph showing the Mean Fluorescence Intensity (MFI) of the Ki67 marker on U87 MG right after the last magnetic field exposure. (a) Column graph of the MFI of the cells in the middle petri vs control (b) Column graph of the MFI of the cells in the bottom petri vs control

From the column graph reported in Figure 3.56, several significant observations can be made.

In contrast to expectations, the mean fluorescence intensity in the middle petri dish is lower than that of the control, but the value is not significantly different from that observed in the bottom petri dish. Interestingly, while the proliferation in the middle petri dish appears unexpectedly high based on the bright-field images, phalloidin and DAPI staining, and the MTT assay, the MFI in the middle petri dish remains only slightly lower than in the bottom petri dish (Figure 3.56b). This result is important, as it suggests that proliferation in the middle petri dish may still be relatively high despite the inhibitory effects observed in other assays. However, further analyses are needed to confirm these findings. Overall, this statistical analysis aligns with the observations made in the confocal microscopy images shown in Figure 3.55.

3.3.4.2 Astrocytes cell line

Finally, the results of the astrocytes cells are presented. In Figure 3.57, the images of the astrocytes cells stained with Ki-67 under the control condition are shown:

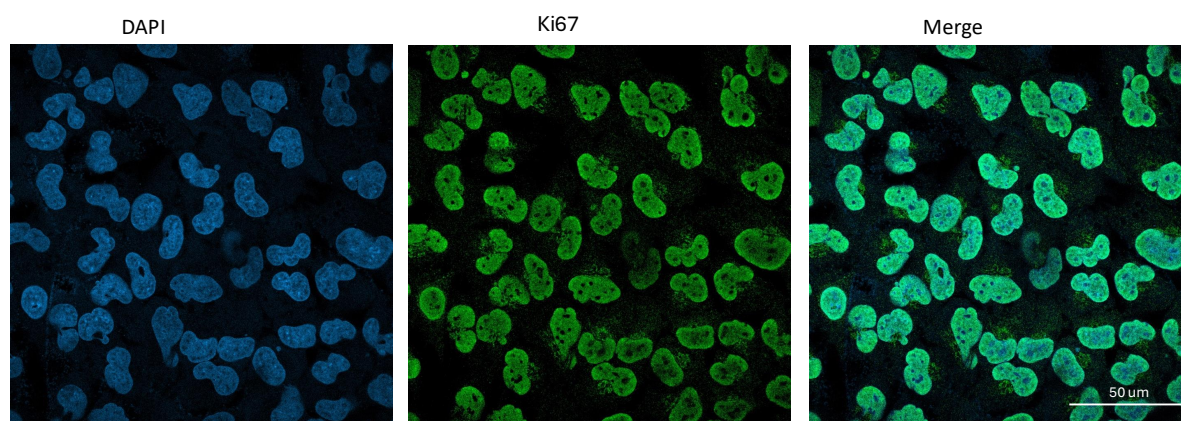


Figure 3.57 Ki-67 staining for proliferation analysis in astrocytes cells. Representative immunofluorescence images of Astrocytes cells under control conditions. DAPI (blue) stains cell nuclei, while Ki-67 (green) marks proliferating cells. The merged images (right column) show the colocalization of nuclei and proliferation marker expression. Scale bars: 20 μm .

It can be observed that the cells in the control condition have regular nuclei, with the Ki-67 marker is well visible and confined within the nuclei, indicating their integrity and healthy condition.

In Figure 3.58, the images of the astrocytes cells exposed to the magnetic fields at the three different intensities are shown.

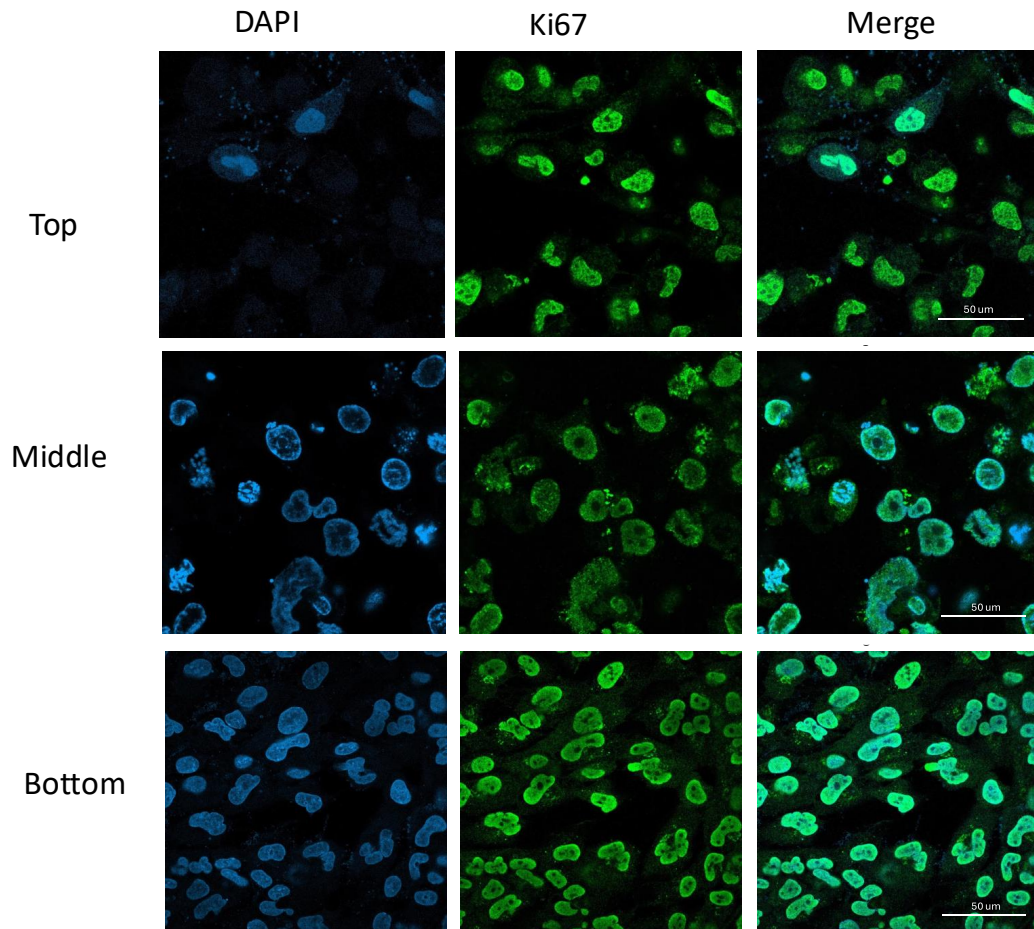


Figure 3.58 Ki-67 staining for proliferation analysis in astrocytes cells. Representative immunofluorescence images of U87 MG cells under control conditions (top row) and after exposure to the electric field (bottom row). DAPI (blue) stains cell nuclei, while Ki-67 (green) marks proliferating cells. The merged images (right column) show the colocalization of nuclei and proliferation marker expression. Scale bars: 20 μm for three different positions within the coil: **Top** (7 mT), **Middle** (6 mT), and **Bottom** (5 mT).

From the image, it is clear that the effects of the field on proliferation differ for each exposed petri dish.

In the top petri dish, the DAPI staining is light, with only a few visible nuclei. This suggests that nuclear integrity has been severely compromised due to exposure. Despite this, Ki-67 is still detectable, indicating that some cells may still be attempting to proliferate.

The cells in the middle petri dish appear severely stressed. Their nuclei exhibit irregular conformations with highly variable shapes. Ki-67 is present, but in some cells, it has relocated to the cytoplasm due to the disruption of the nuclear membrane. As expected, its overall intensity is lower, suggesting a reduced capacity for proliferation.

In the bottom petri dish, the cells appear less stressed compared to those in the middle. Their nuclei maintain a more regular shape. Ki-67 is strongly marked and intense in some cells, indicating that a portion of the surviving population may still be capable of proliferating and increasing their number.

To statically validate the observations, a statistical analysis of the Mean Fluorescence Index (MFI), calculated through Image-J software, was performed and is presented in the column graphs in Figure 3.59.

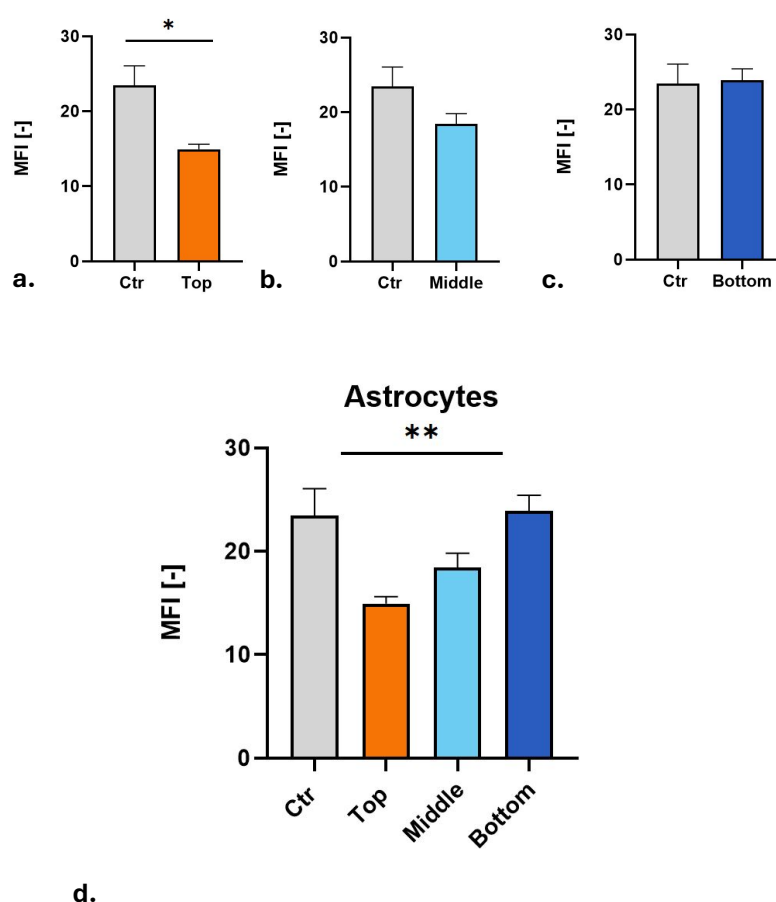


Figure 3.59 Column graphs showing the Mean Fluorescence Intensity (MFI) of the Ki-67 marker on astrocytes cells right after the third magnetic field exposure. (a) Column graph of the MFI of the top petri vs control. (b) Column graph of MFI of the middle petri vs control (c) Column graph of the MFI of the bottom petri vs control. (d) Column graph showing the MFI of all the samples analysed compared to the control. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

In the first column graph (Figure 3.59a), which represents the MFI of the top petri dish, it is evident that the magnetic field significantly inhibited cell proliferation. The difference compared to the controls is statistically significant ($p < 0.05$).

The column graph of the middle petri (Figure 3.59b) shows that the MFI of the exposed cells is lower than that of the control, indicating a reduction in proliferation in this case as well. In contrast, in the column graph comparing the MFI of the bottom petri to the control, the situation is different. The overall signal, and therefore the MFI, is approximately as high as that of the control, suggesting that the magnetic field did not have a concrete effect on cell proliferation. The statistical analysis confirms what was observed in Figure 3.59.

3.3.5 pH variations analysis results

As previously discussed for the electric field in §3.2.5, a similar colour change was also observed following exposure to the magnetic field. During the experiments, this change in the colour of the medium was evident in both astrocytes and U-87 MG cells already after the first exposure, as shown in Figure 3.60.

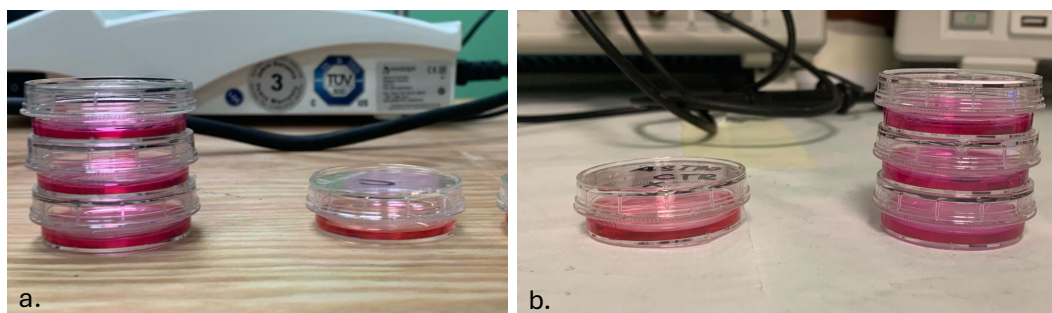


Figure 3.60 Changes in Medium Coloration After Exposure to the Magnetic Field. (a) Photograph of U-87 MG cell cultures: the Petri dishes on the left were exposed to the magnetic field, while the one on the right served as the control. (b) Photograph of astrocyte cell cultures: the Petri dishes on the right were exposed to the magnetic field, while the one on the left served as the control.

It is therefore important to determine whether this change is significant and to investigate whether it is caused by the cells or by some component of the medium.

To assess pH variations, measurements were taken for both the exposed medium, with and without cells, each compared to its respective control group, which was not exposed to the magnetic field.

Again, as previously described in § 3.2.5, statistical analyses were initially performed on the medium without cells, but no significant differences were found. Given the minimal pH variation among the top, middle, and bottom petri dishes, they were considered together in the statistical analysis.

Consequently, the final analysis focused only on the medium containing cells, comparing exposed and non-exposed conditions without considering the different magnetic fields that each individual petri dishes received.

3.3.5.1. U-87 MG cell line

The results of the statistical analysis for the pH variation of U-87 MG are reported in the column graph in Figure 3.61:

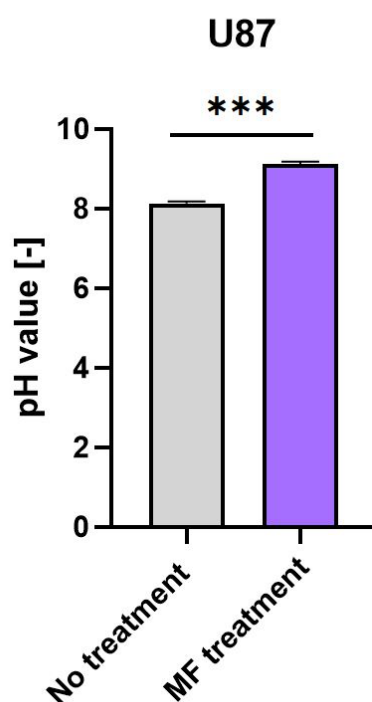


Figure 3.61 Column graph showing the result of the statistical analysis of the pH variation. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

From the graph, it is evident that the pH of the culture medium with cells exposed to the magnetic field has increased compared to the control. The difference is statistically significant ($p < 0.001$). The same consideration regarding the shift to a more basic environment after the exposure done in § 3.2.5.1 can be applied also in this case as well.

3.3.5.2 Astrocytes cell line

The results of the statistical analysis for the pH variation of astrocytes are reported in the column graph in Figure 3.62:

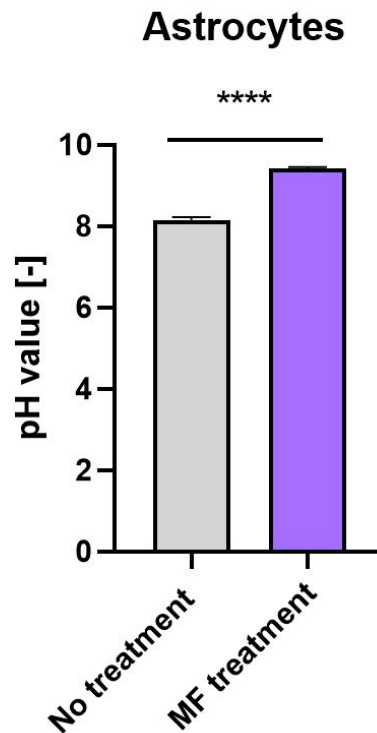


Figure 3.62 Column graph showing the result of the statistical analysis of the pH variation. All values are mean $\pm$ SEM from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control.

As for the U-87 MG cells, the pH of the culture media with astrocyte cells has increased after exposure to the magnetic field, leading to a more basic pH. The difference is statistically significant ($p < 0.0001$). The consideration done for the cellular metabolism and the color switch in §3.2.5.2 are equally valid in this section.

3.3.6 Comparison of the magnetic field effect on the U-87 MG cells and astrocytes

The comparison between the two cell lines, U-87 MG cells and astrocytes, reveals several key differences in their responses to the magnetic field exposure. Observations of the brightfield images show that the strongest inhibitory effects were induced by the highest

magnetic field intensity, applied to the top petri dish for both cell lines. After the last exposure, both cell types were severely stressed and unable to recover even after 24 hours. The response of the two cell lines was quite similar: both detached from the surface after just the first exposure, adopting a rounder shape and losing their original morphology. However, in the middle petri dish, exposed to an intermediate magnetic field intensity, the response differed between the cell lines. While U-87 MG cells exhibited effects similar to those in the top petri, as shown in Figure 3.33, astrocytes displayed a different response. As seen in Figure 3.38, astrocytes in the middle petri were more numerous than U87 MG cells, suggesting a better ability to withstand the stress induced by the magnetic field.

This observation was confirmed by images taken 24 hours after the last exposure: Figure 3.39 shows that astrocytes were recovering and regaining their original shape, whereas U-87 MG cells (Figure 3.34) still appeared stressed, with their growth severely inhibited. Many U-87 MG cells were nearly absent, likely due to detachment and death.

In the bottom petri dish, the response was similar for both cell lines. Both U87 MG cells and astrocytes showed less inhibition compared to the top and middle petri dishes, maintaining a morphology very similar to the control condition. Phalloidin and DAPI staining confirmed the observations from the brightfield for both cell lines. Both U-87 MG cells and astrocytes exhibited similar behaviour, with fragmented F-actin filaments, loss of original morphology, and a significant reduction in cell number. The strongest inhibitory effects of the magnetic field were observed in the top petri dish. However, astrocytes demonstrated a higher capacity to recover from stress, as shown in Figure 3.52, where cells observed 24 hours after the last exposure appeared in better condition compared to U-87 MG cells in Figure 3.47. The most pronounced difference between the two cell lines was observed in the middle petri, where U-87 MG cells were severely inhibited, while astrocytes showed a better capacity for recovery after 24 hours.

MTT assay results indicated that U-87 MG cell viability was lower than that of astrocytes, as shown in the graphs reported in §3.2.3. In both cell lines, the top petri had the lowest viability. However, in the middle petri, U87 MG cells exhibited lower viability than astrocytes, confirming previous observations. The viability of cells in the bottom petri dish for both cell lines was comparable to that of the control condition.

In the top petri, Ki-67 results were not available for U-87 MG cells due to the complete detachment of the cells, which prevented successful staining. However, Ki67 staining for astrocytes was successful and indicated a lower proliferation level compared to the control. In the middle petri, the mean fluorescence intensity (MFI) for U-87 MG cells was slightly lower than the control but unexpectedly higher than anticipated based on the observations from brightfield images, phalloidin and DAPI staining, and MTT assay results. This discrepancy will require further investigation to clarify the underlying cause. Meanwhile, the highest MFI was observed in the bottom petri (Figure 3.56b), despite the weaker magnetic field exposure, which was also associated with the highest Ki-67 levels. For astrocytes, the Ki-67 results for the middle and bottom petri dishes were consistent with the observations made in the brightfield images, as well as the phalloidin and DAPI staining and the MTT assay. : The highest MFI value was recorded in the bottom petri, which closely resembled to the control condition, while the MFI of the middle petri was higher than that of the top petri but lower than the bottom.

The pH variations observed in both cell lines were similar, with a shift to a more basic environment. This suggest that the pH change was not strongly dependent on the cell type, indicating a more general effect of the magnetic field exposure.

In conclusion, both cell lines exhibited stress responses under magnetic field exposure, but astrocytes demonstrated a greater ability to recover, particularly in the middle petri, where U-87 MG cells remained highly inhibited. These findings suggest that astrocytes may possess higher resilience to magnetic field-induced stress, which could be an important factor in understanding their biological response and potential applications in cancer treatments.

Conclusion

The aim of this thesis was to explore the potential of electric and magnetic fields as non-invasive therapeutic approaches for cancer treatment. The first phase involved characterizing the setup used to generate the fields, followed by numerical simulations to verify that the desired intensities and frequencies were being produced. The core of the study focused on evaluating the effects of these fields on glioblastoma cells and astrocytes, with the goal of assessing selective inhibition and understanding changes in cell morphology, viability, proliferation, and metabolism.

To this end, distinct experimental protocols were applied to two cell types: glioblastoma U87-MG cells and human astrocytes, the latter used to model healthy brain tissue. These protocols included phalloidin and DAPI staining, MTT assays, immunofluorescence with Ki-67, and pH measurements.

Exposure to electric field resulted in a significant reduction in cell proliferation and viability. Both U-87 MG cells and astrocytes exhibited stress responses, including morphological changes such as a rounder shape, retraction of cellular projections and detachment from the surface. Notably, U-87 MG cells demonstrated greater initial resilience post-exposure, whereas astrocytes showed more pronounced reductions in viability (§3.2.3). Although some recovery was evident at 24 and 48 hours, cytoskeletal organization remained altered in both cell lines, suggesting lasting structural effects.

In contrast, exposure to magnetic fields revealed that the strongest inhibitory effects occurred at the highest field intensity, with both cell types showing significant stress responses, including detachment from the surface and loss of morphology. However, at intermediate intensities, astrocytes appeared to recover more effectively than U-87 MG cells, which remained highly inhibited (Fig. 3.51). These observations suggest a comparatively greater resilience of astrocytes to magnetic field induced stress.

The analysis of pH did not reveal substantial differences between treated and control conditions. Neither electric nor magnetic field exposure produced significant, cell type-specific changes in pH, indicating that this parameter may not serve as a sensitive readout for the observed stress responses.

Despite promising results, further investigations are needed. Cells exposed to the fields for limited durations appeared capable of recovery; quantifying this process could help

determine which cell type -astrocytes or U-87 MG glioblastoma- plays a more prominent role in regeneration following field-induced stress. Complementary analysis of MTT and Ki-67 expression at later time points (24-48 hours) would provide deeper insight into proliferation capacity, potential damage persistence, and recovery kinetics.

Additional studies should also focus on elucidating the molecular mechanisms underlying the nuclear and cytoskeletal alterations observed after field exposure. Given the close connection between the cytoskeleton and membrane integrity, it would be useful to investigate changes in membrane potential, reactive oxygen species (ROS) production, and DNA alterations using specific assays. Correlating these molecular markers with cellular stress responses and adaptive metabolic shifts may reveal whether electric and magnetic fields enhance drugs uptake or synergize with chemotherapy.

To improve the translational relevance of the findings, future experiments should include 3D cultures, allowing a more physiologically relevant evaluation of cell-cell interactions and field effects across larger cell populations.

Despite the promising findings, several limitations must be acknowledged. A primary technical challenge concerns the coil design used for magnetic field exposure, which currently does not ensure a perfectly uniform field across the entire culture area. This may result in local variations in field intensity and introduce variability in the cellular responses. Additionally, inconsistencies in cell seeding density may have influenced cell-cell interactions and contributed to variability in treatment outcomes.

In conclusion, despite these limitations, the study provided valuable insights into the cellular effects of electric and magnetic fields, highlighting their potential as selective, non-invasive therapeutic tools. These findings support continued investigation and optimization of field-based strategies, which may ultimately contribute to the development of novel therapeutic strategies for cancer treatment.

References

- Arora, M. (2013). Cell Culture Media: A Review. *Materials and Methods*, 3.
- Baldwin, K. T., Murai, K. K., & Khakh, B. S. (2024). Astrocyte morphology. In *Trends in Cell Biology* (Vol. 34, Issue 7, pp. 547–565). Elsevier Ltd.
- Baskin, D. S., Sharpe, M. A., Nguyen, L., & Helekar, S. A. (2021). Case Report: End-Stage Recurrent Glioblastoma Treated with a New Noninvasive Non-Contact Oncomagnetic Device. *Frontiers in Oncology*, 11.
- Berkelmann, L., Bader, A., Meshksar, S., Dierks, A., Hatipoglu Majernik, G., Krauss, J. K., Schwabe, K., Manteuffel, D., & Ngezahayo, A. (2019). Tumour-treating fields (TTFields): Investigations on the mechanism of action by electromagnetic exposure of cells in telophase/cytokinesis. *Scientific Reports*, 9(1).
- Cai, Z., Yang, Z., Wang, Y., Li, Y., Zhao, H., Zhao, H., Yang, X., Wang, C., Meng, T., Tong, X., Zheng, H., He, Z., Niu, C., Yang, J., Chen, F., Yang, Z., Zou, Z., & Li, W. (2024). Tumor treating induced fields: a new treatment option for patients with glioblastoma. *Frontiers in Neurology*, 15.
- Chang, E., Patel, C. B., Pohling, C., Young, C., Song, J., Flores, T. A., Zeng, Y., Joubert, L. M., Arami, H., Natarajan, A., Sinclair, R., & Gambhir, S. S. (2018). Tumor treating fields increases membrane permeability in glioblastoma cells. *Cell Death Discovery*, 4(1).
- Davies, A. M., Weinberg, U., & Palti, Y. (2013). Tumor treating fields: A new frontier in cancer therapy. *Annals of the New York Academy of Sciences*, 1291(1), 86–95.
- Dennise Villanueva, M. M. (2013). Exploring the impact of static magnetic fields on human glioblastoma cells in vitro.
- Fabian, D., Eibl, M. del P. G. P., Alnahhas, I., Sebastian, N., Giglio, P., Puduvalli, V., Gonzalez, J., & Palmer, J. D. (2019a). Treatment of glioblastoma (GBM) with the addition of tumor-treating fields (TTF): A review. In *Cancers* (Vol. 11, Issue 2). MDPI AG.

- Gartzke, J., & Lange, K. (2002). Downloaded from journals.physiology.org/journal/ajpcell at Università di Padova. *Am J Physiol Cell Physiol*, 283, 1333–1346.
- Giladi, M., Munster, M., Schneiderman, R. S., Voloshin, T., Porat, Y., Blat, R., Zielinska-Chomej, K., Hååg, P., Bomzon, Z., Kirson, E. D., Weinberg, U., Viktorsson, K., Lewensohn, R., & Palti, Y. (2017). Tumor treating fields (TTFields) delay DNA damage repair following radiation treatment of glioma cells. *Radiation Oncology*, 12(1).
- Guo, X., Yang, X., Wu, J., Yang, H., Li, Y., Li, J., Liu, Q., Wu, C., Xing, H., Liu, P., Wang, Y., Hu, C., & Ma, W. (2022). Tumor-Treating Fields in Glioblastomas: Past, Present, and Future. In *Cancers* (Vol. 14, Issue 15). MDPI.
- Kim, E. H., Song, H. S., Yoo, S. H., & Yoon, M. (2016a). Tumor treating fields inhibit glioblastoma cell migration, invasion and angiogenesis (Vol. 7, Issue 40).
- Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? In *Trends in Biochemical Sciences* (Vol. 41, Issue 3, pp. 211–218). Elsevier Ltd.
- Liu, Y., Tang, Q., Tao, Q., Dong, H., Shi, Z., & Zhou, L. (2024a). Low frequency magnetic field therapy for glioblastoma: Current advances, mechanisms, challenges and future perspectives. In *Journal of Advanced Research*. Elsevier B.V.
- Ozdil, B., Calik-Kocaturk, D., Altunayar-Unsalan, C., Acikgoz, E., Oltulu, F., Gorgulu, V., Uysal, A., Oktem, G., Unsalan, O., Guler, G., & Aktug, H. (2024). Differences and similarities in biophysical and biological characteristics between U87 MG glioblastoma and astrocyte cells. *Histochemistry and Cell Biology*, 161(1), 43–57.
- Pei, Z., Lee, K. C., Khan, A., Erisnor, G., & Wang, H. Y. (2020). Pathway analysis of glutamate-mediated, calcium-related signaling in glioma progression. In *Biochemical Pharmacology* (Vol. 176). Elsevier Inc.
- Salvatore, J. R. (2014). Electromagnetic Fields. *Encyclopedia of Toxicology: Third Edition*, 316–319.

- Schlieper-Scherf, S., Hebach, N., Hausmann, D., Azorín, D. D., Hoffmann, D. C., Horschitz, S., Maier, E., Koch, P., Karreman, M. A., Etminan, N., & Ratliff, M. (2024). Disrupting glioblastoma networks with tumor treating fields (TTFields) in in vitro models. *Journal of Neuro-Oncology*. <https://doi.org/10.1007/s11060-024-04786-0>
- Sun, J., Tong, Y., Jia, Y., Jia, X., Wang, H., Chen, Y., Wu, J., Jin, W., Ma, Z., Cao, K., Li, X., Chen, Z., & Yang, G. (2023). Effects of extremely low frequency electromagnetic fields on the tumor cell inhibition and the possible mechanism. *Scientific Reports*, 13(1)
- Tan, A. C., Ashley, D. M., López, G. Y., Malinzak, M., Friedman, H. S., & Khasraw, M. (2020). Management of glioblastoma: State of the art and future directions. *CA: A Cancer Journal for Clinicians*, 70(4), 299–312
- Teodori, L., Albertini, M. C., Uguccioni, F., Falcieri, E., Rocchi, M. B. L., Battistelli, M., Coluzza, C., Piantanida, G., Bergamaschi, A., Magrini, A., Mucciato, R., & Accorsi, A. (2006). Static magnetic fields affect cell size, shape, orientation, and membrane surface of human glioblastoma cells, as demonstrated by electron, optic, and atomic force microscopy. *Cytometry Part A* 69(2), 75–85.
- Tofani, S., Barone, D., Cintorino, M., De Santi, M. M., Ferrara, A., Orlassino, R., Ossola, P., Peroglio, F., Rolfo, K., & Ronchetto, F. (2001). Static and ELF magnetic fields induce tumor growth inhibition and apoptosis. *Bioelectromagnetics*, 22(6), 419–428.
- Urbanska, K., Sokolowska, J., Szmidt, M., & Sysa, P. (2014). Glioblastoma multiforme - An overview. In *Wspolczesna Onkologia* (Vol. 18, Issue 5, pp. 307–312). Termedia Publishing House Ltd.
- Voloshin, T., Schneiderman, R. S., Volodin, A., Shamir, R. R., Kaynan, N., Zeevi, E., Koren, L., Klein-Goldberg, A., Paz, R., Giladi, M., Bomzon, Z., Weinberg, U., & Palti, Y. (2020). Tumor treating fields (Ttfields) hinder cancer cell motility through regulation of microtubule and actin dynamics. *Cancers*, 12(10), 1–18.

Xu, A., Wang, Q., Lv, X., & Lin, T. (2021). Progressive Study on the Non-thermal Effects of Magnetic Field Therapy in Oncology. In *Frontiers in Oncology* (Vol. 11). Frontiers Media S.A.

Zhang, L., Ji, X., Yang, X., & Zhang, X. (n.d.). Cell type-and density-dependent effect of 1 T static magnetic field on cell proliferation. www.impactjournals.com/oncotarget