*ORIGINAL ARTICLE*

Annals of Agricultural and Environmental Medicine 2023, Vol 30, No 4, 763–772
www.aaem.pl

BY-NC

# Effects of 2.4 GHz radiofrequency electromagnetic field (RF-EMF) on glioblastoma cells (U -118 MG)

**Agnieszka Nowak-Terpiłowska[1,A,C-F], Romuald Górski[1,A-B,D], Małgorzata Marszałek[1,B], Stanisław Wosiński[2,A-B], Rafał Przesmycki[3,B-C], Marek Bugaj[3,B-C], Leszek Nowosielski[3,B-C], Mikołaj Baranowski[4,B-C], Joanna Zeyland[1,B]**

[1] *University of Life Sciences, Poznań, Poland*
[2] *ADR Technology, Poznań, Poland*
[3] *Military University of Technology, Warsaw, Poland*
[4] *Adam Mickiewicz University, Poznań, Poland*
*A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article*

Nowak-Terpiłowska A, Górski R, Marszałek M, Wosiński S, Przesmycki R, Bugaj M, Nowosielski L, Baranowski M, Zeyland J. Effects of 2.4 GHz radiofrequency electromagnetic field (RF-EMF) on glioblastoma cells (U-118 MG). Ann Agric Environ Med. 2023; 30(4): 763–772. doi: 10.26444/aaem/176405

## Abstract

**Introduction.** Mobile phones and Wi-Fi are the most commonly used forms of telecommunications. Initiated with the first generation, the mobile telephony is currently in its fifth generation without being screened extensively for any biological effects that it may have on humans or on animals. Some studies indicate that high frequency electromagnetic radiation emitted by mobile phone and Wi-Fi connection can have a negative effect upon human health, and can cause cancer, including brain tumour.

**Objective.** The aim of the study was to investigate the influence of 2.4 GHz radiofrequency electromagnetic field (RF-EMF) on the proliferation and morphology of normal (human embryonic kidney cell line Hek-293) and cancer cells (glioblastoma cell line U-118 MG).

**Materials and method.** The cell cultures were incubated in RF-EMF at the frequency of 2.4 GHz, with or without dielectric screen, for 24, 48 and 72h. In order to analyse the influence of the electromagnetic field on cell lines, Cytotoxicity test Cell Counting Kit-8 was performed. To protect cells against emission of the electromagnetic field, a dielectric screen was used.

**Results.** It was found that 2.4 GHz RF electromagnetic field exposure caused a significant decrease in viability of U-118 MG and Hek-293 cells. The impact of the electromagnetic field was strongest in the case of cancer cells, and the decrease in their survival was much greater compared to the healthy (normal) cells of the Hek-293 line.

**Conclusions.** Results of the study indicate that using a radio frequency electromagnetic field (2.4 GHz) has a clearly negative effect on the metabolic activity of glioblastoma cells. RF-EMF has much less impact on reducing the viability of normal cells (Hek -293) than cancer cells.

## Key words

proliferation, morphology, glioblastoma cancer cells, human embryonic kidney cells, radio dielectric screen, radiofrequency electromagnetic field

## INTRODUCTION

Mobile telecommunication devices, such as mobile phones and Wi-Fi, have become seamlessly integrated into the fabric of present-day telecommunications. The omnipresence of mobile phones transcends global demographics, exhibiting a high prevalence among individuals of diverse age groups, thus raising potential public health concerns. The adoption of mobile phones has shown exponential growth, as evidenced by the global count of over five billion individual mobile phone subscriptions in December 2017, with nearly three-quarters of the world's population possessing a mobile subscription in 2020 [1].

The advent of 5G technologies operating on electromagnetic waves, has evoked the prospect of hyper-connected network environments capable of supporting augmented reality and virtual reality applications. The technology utililzes two main frequency bands – 3.5 GHz and 28 GHz. While the influence of the 3.5 GHz band on human physiology is akin to its predecessor, 4G, and leverages existing base stations, the 28 GHz band poses distinctive implications for human health, necessitating the installation of base stations in closer proximity to users. Consequently, the long-term health effects of 5G exposure on infants and children remain unclear, as comprehensive evaluations of the 5G impact on the young generation have not yet been undertaken [2].

Of note, mobile phone users are perpetually subjected to radiation emanating from their devices, even when the phone is not in active use. Additionally, the human body endures continuous electromagnetic (EM) exposure stemming from mobile communication infrastructures, especially from base station towers [3]. The proliferation of wireless network technologies, including wireless local area network (WLAN) hotspots and Wi-Fi networks, also escalates the potential

✉ Address for correspondence: Agnieszka Nowak-Terpiłowska, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland
E-mail: agnieszka.terpilowska@up.poznan.pl

Received: 19.10.2023; accepted: 05.12.2023; first published: 21.12.2023

for excessive radiation exposure in the radiofrequency EM spectrum [4]. Research has disclosed elevated EM radiation levels in urban areas across different countries [5].

Mobile phones and Wi-Fi base stations emit electromagnetic fields characterized by radiofrequencies which harbour the potential for carcinogenicity in humans. The International Agency for Research on Cancer (IARC) at the World Health Organization (WHO) has ascribed a 'possible human carcinogen' classification (Group 2B) to radiofrequency level electromagnetic fields (RF-EMF), which include radiation from mobile devices and other non-ionizing EMF-emitting apparatus [6]. The usage of mobile phones has been linked to an increased risk of brain tumours, such as glioma, meningioma, and acoustic neuroma, owing to the proximity of the brain to the EMF radiation source during phone calls. The initial report on the association between mobile phone usage and increased brain tumour risk was published approximately twenty years ago [7]. Subsequently, extensive research has corroborated the impact of mobile phones on the incidence of malignant and benign brain tumours [8, 9, 10, 11]. However, a large international case-control study (INTERPHONE study, 2000) aimed at elucidating the connection between adult brain tumour risk and mobile phone utilization, failed to identify a pervasive increase in brain tumour incidence. Nevertheless, among individuals in the 10th highest decile of cumulative call time (≥1,640 hours), elevated odds ratios of 1.4 for glioma and 1.15 for meningioma were recorded [12].

It is imperative to acknowledge the fact that radiation exposure from mobile phones predominantly affects the region of the brain proximate to the ear, particularly the temporal lobe, situated on the side of the head where the phone is customarily held. A substantial portion of radiofrequency (RF) energy, ranging from 97% – 99%, is absorbed by the hemisphere of the brain corresponding to the phone's location, with a significant fraction (50–60%) concentrating in the temporal lobe [13].

The adverse effects of electromagnetic radiation from mobile phones are more conspicuous in younger individuals. Research by Hardell et al. revealed a 5.2-fold elevated risk of malignant brain tumours after just one year of mobile phone usage in individuals under 20 years of age, compared to an odds ratio of 1.4 for all age groups [14]. Furthermore, children are found to absorb twice as much RF energy from mobile phones as adults owing to their smaller brain size, thinner ear pinna, less protective skin, and thinner skull, which facilitates deeper penetration of RF energy into the developing brain [15, 16]. Acoustic neuroma (vestibular schwannoma), a nerve sheath tumour, attracts specific attention in the context of mobile phone usage due to RF energy accumulating in the vicinity of the eighth cranial nerve, where acoustic neuromas typically originate.

Several studies have elucidated the links between mobile phone usage and parotid gland tumours [17], non-Hodgkin's lymphoma [18, 19], and breast cancer [9]. The exposure to microwaves in the radiofrequency EM spectrum during mobile phone usage may also elevate risks related to testicular cancer, particularly when the phone is stored in close proximity to the testes [20].

The genesis of cancerous cells in this context is attributed to DNA damage incurred from the effects of high-frequency electromagnetic fields. The precise mechanisms underlying DNA alterations in response to RF-EMF exposure have not been exhaustively investigated. While the energy levels associated with RF-EMF exposure are insufficient to induce direct DNA strand breaks or crosslinks, it is inferred that DNA damage arises from cellular biochemical processes, including the generation of free radicals. Numerous studies have demonstrated the augmentation of free radical activity in response to RF-EMF exposure [21, 22]. RF-EMF exposure is implicated in the development of oxidative base damage in mouse spermatocyte-derived cell lines, mediated through the generation of reactive oxygen species (ROS) [21, 22].

RF exposure may induce various lesions in the brain, not all of which are associated with malignancy. Prenatal exposure of rats to 900 MHz RF radiation results in a reduction in the number of granule cells within the dentate gyrus, implying that RF-EMF impacts the proliferation of these cells in the rat hippocampus. Such cell loss may be attributable to an inhibition of granule cell neurogenesis within the dentate gyrus [23]. Subsequent post-natal exposure of rats to 900 MHz RF radiation induces a significant reduction in the number of pyramidal cells within the cornu ammonis [24].

Mice exposed to RF from mobile phones exhibit hyperactivity and memory impairment, potentially mediated by impaired glutamatergic synaptic transmission onto layer V pyramidal neurons within the prefrontal cortex [25, 26]. Research findings indicate substantial evidence ($p < 0.002$) for neuronal damage in the cortex, hippocampus, and basal ganglia of rats exposed to RF radiation from mobile phones, a phenomenon linked to the leakage of albumin across the blood-brain barrier (BBB) [25, 26]. Nittby et al. also observed alterations in the permeability of the blood-brain barrier induced by electromagnetic radiation from mobile phones, leading to albumin extravasation both immediately and 14 days following two hours of exposure [27]. Furthermore, other studies on rats suggest that RF-EMF exposure may induce oxidative stress within the brain [28], a phenomenon constituting a putative biological mechanism of RF-EMF effects.

The results of the studies conducted on rats revealed that long-term exposure of 2.4 GHz Wi-Fi radiation can alter the expression of some of the micro-RNAs in brain tissue [29], and may change deoxyribonucleic acid methylation, histone modification, and chromatin remodelling [30].

In the light of the above threats, the ideal solution would be to create a screen matched with the characteristics to suppress these harmful fields. EMF shielding is required in many cases to reduce its negative effects, especially in the case of cells and tissue vulnerable to DNA damage.

The aim of the presented *in vitro* study was to investigate the influence of RF-EMF on normal and cancer human cells. The level of cell proliferation and their morphology after a specific time of cell cultures placed within the electromagnetic field were examined. At the same time, the effectiveness of the dielectric screen in absorbing the radiation emitted was also examined.

## MATERIALS AND METHOD

**Cell cultivation.** Cell cultures of the glioblastoma cell line (U-118 MG, ATCC® HTB-15™) and human embryonic kidney cell line (Hek-293 ATCC®, CRL-1573™) were cultivated. The base medium used in the cell culture was Dulbecco's Modified Eagle's Medium (DMEM). The complete medium consisted of foetal bovine serum (FBS) to a final concentration of

10%, nutrient mixture F-12 to a final concentration of 40%, and a mixture of antibiotics (penicillin, streptomycin, amphotericin) to a final concentration of 1%. Cells were grown in 25 $cm^2$ culture vessels at 37 °C in an atmosphere containing 5% $CO_2$ (New Brunswick S41i Incubator, Eppendorf). For passage, cells were briefly washed with Hank's Balanced Salt Solution to remove residual FBS, and then incubated with 750 µl of 0.25% trypsin-EDTA solution for 10 minutes at 37 °C. After reaching the appropriate level of detachment of cells, evaluated using an inverted microscope, the culture vessel was replenished with fresh culture medium and poured into two new vessels. Changes of the culture medium took place, on average, every two or three days until the cells achieved a confluence of 80%. Due to the adherent nature of both cell lines, the cultivation conditions were the same.

**Cytotoxicity test Cell Counting Kit-8.** In order to analyse the influence of the electromagnetic field on cell lines, experiments were carried out according to the following scheme: cells were grown under standard conditions (DMEM, F-12, antibiotic, FBS) until they reached 80% confluency. After reaching the appropriate amount of confluence, the cell suspensions were transferred to multi-well vessels (96-well plate, 5000 cells/100 µl) and incubated for 24 hours.

**Electromagnetic radiation measurement.** Before the experiment, measurements of electromagnetic radiation inside the device connected to the network were carried out using the Trifield TF2. It turned out that inside the chamber the power density was 0.35 $mW/m^2$. Due to the generation of electromagnetic radiation by the incubator connected to the network, it was necessary to use a dielectric screen in control combinations in order to absorb the EMF generated by the device. The best results were achieved by placing a dielectric on both the top and bottom of the control cell culture plate. The power density then dropped to only 0.01 $mW/m^2$ (Tab. 1). For comparison, in combinations with the use of a high-frequency radiation generator (Wi-Fi) and an EMF-absorbing screen placed on the bottom and top of the tile, the power density dropped from 20 $mW/m^2$ to as little as 0.3 $mW/m^2$, and with the use of a screen only from the bottom – 3.5 $mW/m^2$. Bearing the results in mind, double-sided shielding of the cell culture was used in the research.

**Table 1.** Power density

| Lp. | Type of screening | $mW/m^2$ |
|---|---|---|
| 1 | Chamber with Wi-Fi | 20 |
| 2 | Chamber with Wi-Fi screened from the bottom | 3.5 |
| 3 | Chamber with Wi-Fi screen from the top and bottom | 0.3 |
| 4 | Chamber without Wi-Fi | 0.35 |
| 5 | Control chamber without Wi-Fi + screened from the top and bottom | 0.01 |

Typical electric field shields use conductive media which needs grounding: metal layers, polymer or glass covered metal nets, conductive polymers, cotton/polyester blended with microscopic silver or steel fibres.

A co-author of this study [31, 32] has developed an advanced technology – a nanocomposite of Advance Dielectric Radiation Trap (ADR) and TEX, utilizing ADR Technology which causes the electric component of electromagnetic radiation to be absorbed by water, which is confined and dispersed in a variety of ways to form a dielectric matrix.

The basic matrix is woven from three kinds of yarn or threads: Rayon (viscose), Nylon (polyamide 66) and Schoeller thread (PES yarn with 20% metallic filing) in accord with the weight ratio of 44:36:20. After weaving (using broken twill) the fabric was saturated with ADR Sol, and after it had dried had a weight of 110 $g/m^2$.

X-ray energy dispersive spectroscopy (EDS) was utilised to obtain information on the distribution of the different elements of the ADR TEX shield. All kinds of threads (viscose, PA 66 and PES) are built of carbon atoms which are visible in the microanalytical image as having green contrast. In Figure 1, EDS shows green contrast of carbon atoms in viscose rayon, PA 66 and PES together with the magenta, yellow and blue contrast of chromium, iron and nickel atoms in the Schoeller yarn.

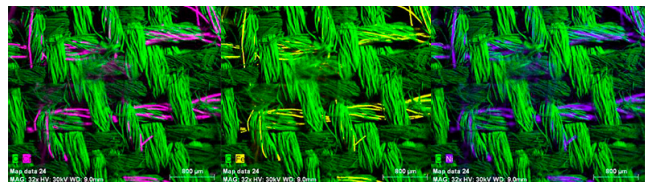


**Figure 1.** EDS imaging of carbon C in (Rayon, PA 66 and PES) and chromium Cr (magenta), iron Fe (yellow) and nickel Ni (blue) in ADR TEX shield

To obtain information on the origin of the intrinsic dielectric absorption of ADR TEX shield, frequency dependence of tan δ of a fabric woven from two kinds of threads was measured: Rayon (viscose) and Nylon (polyamide 66) in weight ratio of 55:45 without the Schoeller thread (PES yarn with 20% metallic filing). After weaving (broken twill weave), the fabric was soaked with ADR Sol, dried in air. Figure 2 shows the dispersion of tan δ of an EMF shield made from Rayon/Nylon 55/45 fabric with ADR Technology. A broad maximum centred at ~1 kHz can be observed, due to water dispersed in the Rayon/Nylon/ ADR Sol composite. Thus, dielectric absorption in the frequency range 10 kHz – 1 MHz of the ADR TEX shield (Fig. 3) is an intrinsic feature related to the presence of the Schoeller thread (PES yarn with 20% Cr/Fe/Ni filing).

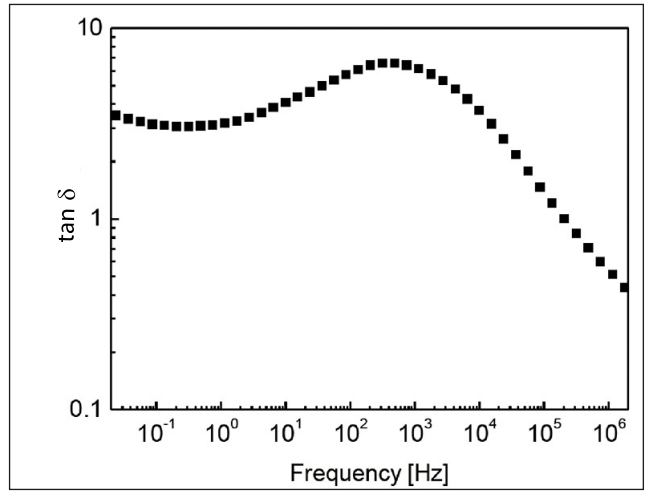



**Figure 2.** Room temperature frequency dependence of tan δ for a sample of Rayo/Nylon 55/45 fabric shield made with ADR Technology

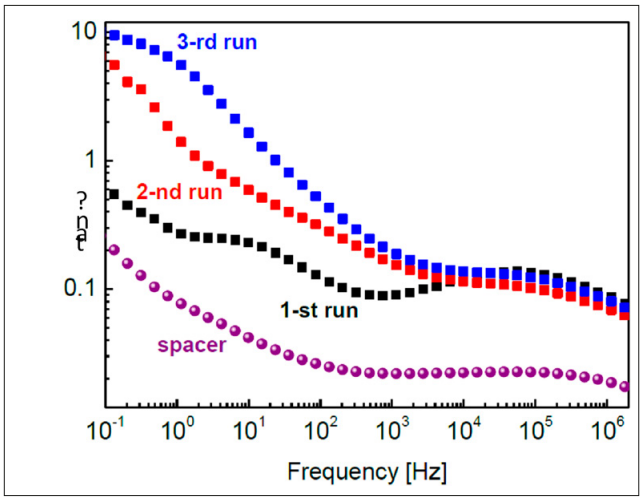



**Figure 3.** Room temperature frequency dependence of tan δ for a sample of ADR TEX shield placed between two dielectric spacers. Tan δ dispersion of the dielectric spacer is also apparent

The ADR TEX exhibits high dielectric absorption in the low-frequency range and does not need grounding and is also the screen electromagnetic HF by reflection (Fig. 4 and 5).

Each of the tested cell lines (cancer cells and normal cells) were exposed to an electromagnetic field with a frequency of 2.4 GHz emitted by a device specially designed for the purpose, in two variants: with and without a dielectric screen. In addition, the metabolic activity of cells without the electromagnetic field generated by the emitter, and without the screen, was tested in order to determine the impact of radiation generated inside the incubator connected to the network. The control cell lines were cultivated in conditions without the influence of RF-EMF generated by emitter but used the screen, which absorbed the radiation generated by the incubator. The experiment was conducted for 72 hours. Cell viability was measured after 24, 48 and 72 hours of incubation. To analyse changes in cell viability, CCK-8 – Cell Counting Kit – 8 (Sigma) was performed. The number of live (metabolically active) cells is proportional to the amount of formazan produced. This, in turn, is produced by the reduction of tetrazolium salts through the cellular dehydrogenase. After the specified cultivation time (24, 48, 72 hours), CCK-8 reagent was added to selected samples. Absorbance readings were made at 450 nm in the ELx808 plate reader (BioTek). The viability of test samples was defined

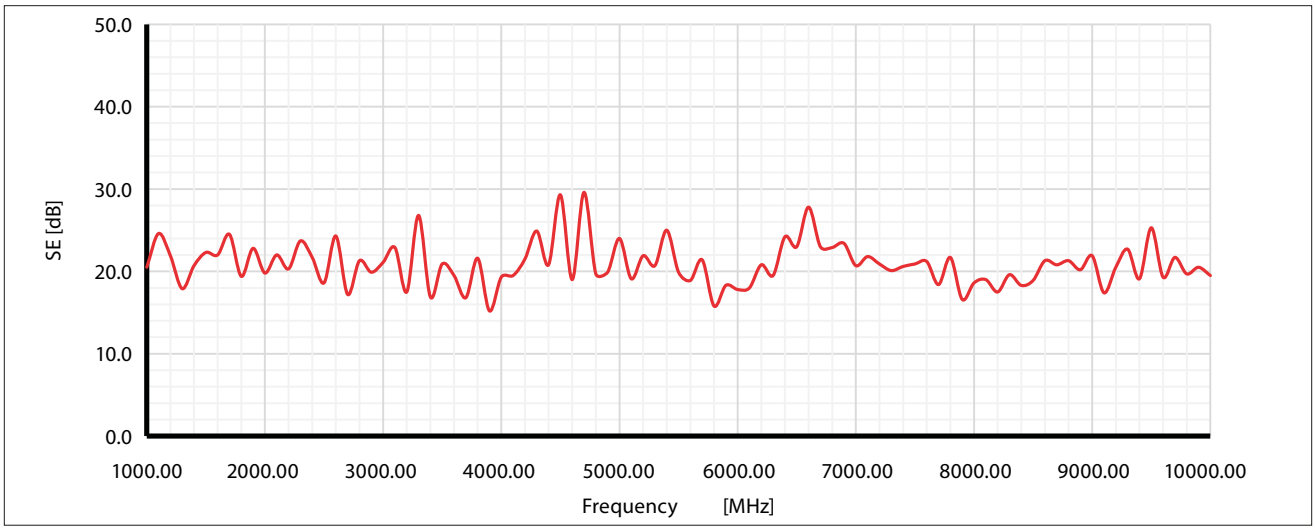



**Figure 4.** Shielding efficiency of fabric ADR TEX [dB] Vertical polarization

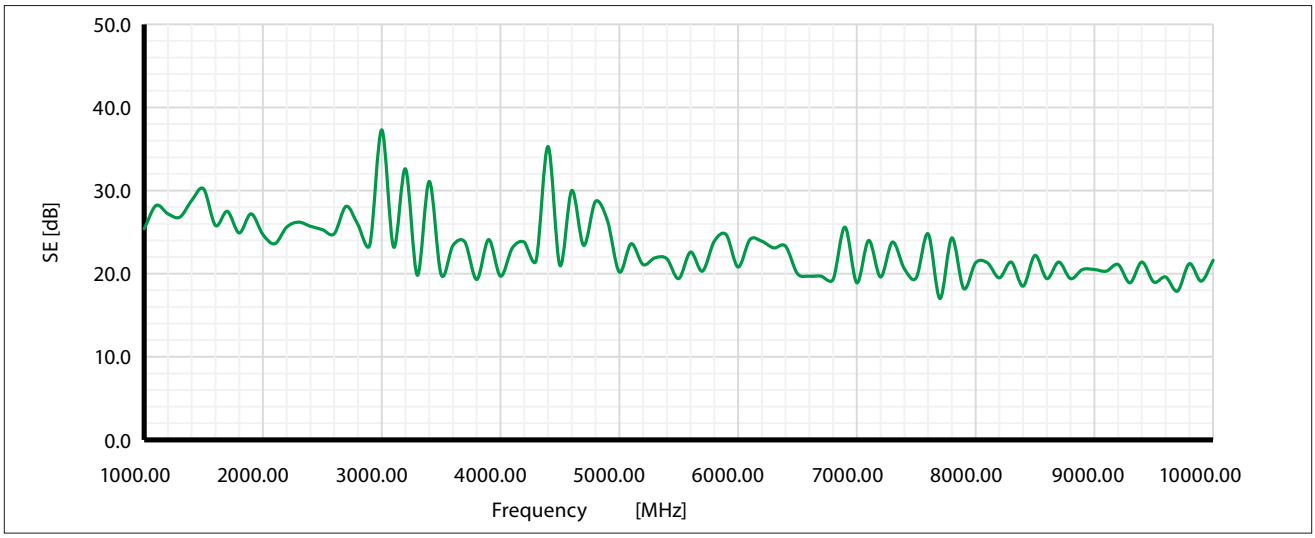



**Figure 5.** Shielding efficiency of fabric ADR TEX [dB] Horizontal polarization

as the percentage of control samples viability, determined as 100% in each time, respectively. All variants of the experiment were performed in triplicate for both cell lines.

**Analysis of changes in cell morphology.** The study of changes in cell morphology of both cell lines was performed using the Axiovert 200 inverted light microscope (Zeiss) and ZOE Fluorescent Cell Imager device (Bio-Rad). Images of cells were taken after 1, 24, 48 and 72 hours, respectively. Morphology analysis was performed for both test samples (exposure to the electromagnetic field), samples with and without a dielectric screen and control samples (free from the influence of RF-EMF).

**Description of the hardware part – an electromagnetic field generator.** The number of devices with radio transmitters around us is steadily increasing. We are thus subjected to increasing exposure to radio waves and it is increasingly difficult to find a place without coverage, especially in cities. In an increasing number of houses, not only Wi-Fi but also domotics and all kinds of automation devices, are being constructed. The transmission of information via wires is abandoned in favour of wireless technology. A large number of commercially available devices contain basic IoT nodes. Such devices connect, for example, to the cloud using a local Wi-Fi network.

Considering the above, it was decided to choose ESP systems and a device that could be both a basic IoT node and an independent, fully configurable router. This is a small module that allows connection with, for example, Arduino to a wireless network, which is widely popular among electronics and new technology enthusiasts. It can work as a standalone system with sensors connected to GPIO ports and has a relatively large memory for 1MB Flash software. Recently, the ESP8266 revolutionized the world of IoT with its entry into the market, quickly setting its own standards. The reasons for the popularization of this module can be found in the low price, huge possibilities resulting from a processor powerful by the standards of microcontrollers and built-in Wi-Fi support. The module itself is less than 2.5 cm long and is equipped with a very powerful processor and 2 GPIO ports supporting PWM. In addition, for research purposes, systems with an antenna with a well-defined directionality of radiation were sought. Antenna simulations were carried out for the purposes of work [1].

The module will not work without the appropriate software. There are many ways to programme ESP. First, the manufacturer provides an official SDK, and there is also an alternative community-created SDK called ESP-Open-SDK. In addition, other solutions are available that allow the creation of applications in C++, Lua, and even MicroPython. ESP can also be treated as ordinary communication modules with which information is exchanged using AT commands – these are also used to configure the device.

The list of available tools for developers is also described on Wikipedia, many examples can also be found on the manufacturer's website. However, most people use two methods. The first one is programming the ESP8266 in the Arduino environment. After installing the appropriate libraries, programmes can be written on the ESP, the same as on publicly available Arduino modules. The second popular option of using ESP modules is the use of ready-made ESP Easy batches, which allow use of the the module's peripherals from the panel available through a standard web browser (the module then becomes a server).

For the purposes of this study, the software was created in the Arduino environment. The device was configured to automatically connect to the local Wi-Fi network configured on the router. The design of the experimental setup is shown in Figure 6.

When creating the sets, efforts were made to simulate the communication conditions of the sensors with the network as correctly as possible. Therefore, the protocol included sending information from the ESP modules via the local Asus RT N12, 2.4GHz router, for example, to the cloud. The method of communication between the test stands and the router is shown in Figure 7.

ESP operating parameters, such as the output power of the radio power amplifier +19.5dBm and the network standard 802.11b mode, are configured rigidly in the firmware. The operating frequency of the device was around 2.4 GHz. Detailed frequency information in the function of channel number is shown in Table 1. The transceiver for radio frequency supports a total of 14 channels, aligning with the standards set by IEEE802.11b/g/n. The 2.4 GHz transmitter converts quadrature baseband signals to 2.4 GHz, driving the antenna through a high-power CMOS power amplifier. Enhanced linearity of the power amplifier is achieved through the digital calibration function, resulting in cutting-edge performance that achieves an average TX power of +19.5

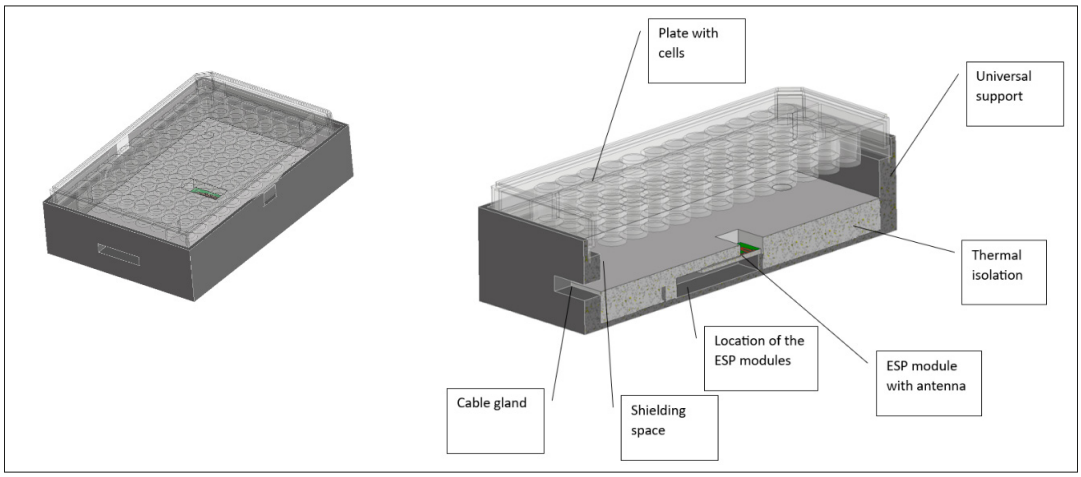



**Figure 6.** Designed experimental setup with universal support printed using PLA 3D printer

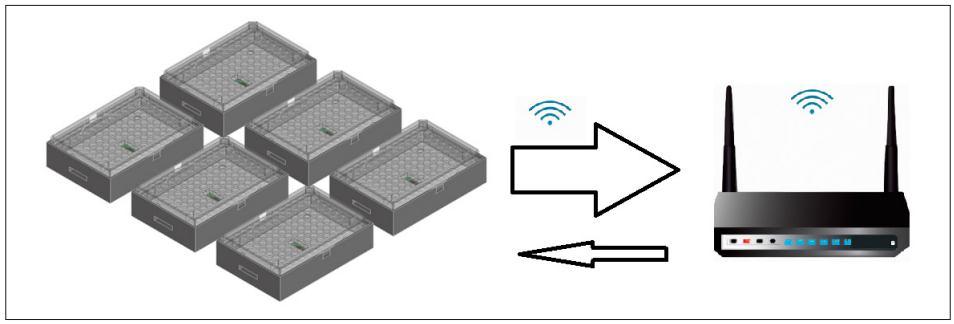


**Figure 7.** Schematic diagram of communication between ESP modules and the router

dBm for 802.11b transmission and +18 dBm for 802.11n (MSC0) transmission. ESP8266EX incorporates the TCP/IP protocol and the comprehensive 802.11 b/g/n WLAN MAC protocol. Within the Distributed Control Function (DCF), it supports Basic Service Set (BSS) STA and SoftAP operations. The power management is efficiently handled with minimal intervention from the host to reduce the active-duty period [2].

**Table 2.** ESP frequency channel

| Channel No. | Frequency (MHz) | Channel No. | Frequency (MHz) |
|---|---|---|---|
| 1 | 2412 | 8 | 2447 |
| 2 | 2417 | 9 | 2452 |
| 3 | 2422 | 10 | 2457 |
| 4 | 2427 | 11 | 2462 |
| 5 | 2432 | 12 | 2467 |
| 6 | 2437 | 13 | 2472 |
| 7 | 2442 | 14 | 2484 |

The packet size was defined as 4095. Serial communication was used to control the correctness of the connection – the popular RS232 port. Thanks to this, after connecting to a computer, it was possible to follow the process of connecting to the router and data exchange on an ongoing basis. For simplicity, UDP was the communication protocol over Wi-Fi for data exchange.

The spectrum analyser GWinstek GDS-3504 was used to measure the maximum power emitted by the ESP device by soldering onto the pins just in front of the antenna. The modules were powered from 5V switching power supplies via the micro-USB connector. For the purposes of the study, 30 sets were assembled and connected to two routers.

Due to the heating of the modules, special spacers made of dense polystyrene were used between the modules and the plates on which the cells were grown. This completely reduced the uncontrolled increase in temperature in the cells.

**Statistical analysis.** The survival results of U-118 MG and Hek-293 cells for three different culture times (24h, 48h, 72h) were subjected to statistical analysis. Mean values were tested for normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene test for homogeneity of variance across multiple runs). To compare the mean values, the t-test was used, for which the following values were adopted: $p < 0.001$ (*** confidence level 0.999), $p < 0.01$ (** confidence level 0.99), and $p < 0.05$ (* confidence level 0.95).

## RESULTS

The influence of RF-EMF on U-118 MG and Hek-293 cells is presented in Figure 8. The electromagnetic field exposure caused a significant decrease viability of U-118 MG and Hek-293 cells in the following days of observation, compared to the control group. The greatest decrease occurred after 72 hours of incubation. The impact of the electromagnetic field was strongest in the case of cancer cells, and the decrease in their survival was much greater in comparison with the healthy (normal) cells of the Hek -293 line. There was no significant effect of the use of a dielectric screen on the survival of the tested cells. It should be noted that this screen effectively absorbed the electromagnetic radiation emitted by the cell culture incubator connected to electrical network. Cell viability in control combinations without the use of RF field, protected with a dielectric screen, was significantly higher compared to the combination without an electromagnetic field and screen. This was the case with both U-118 MG and Hek-293 cells.

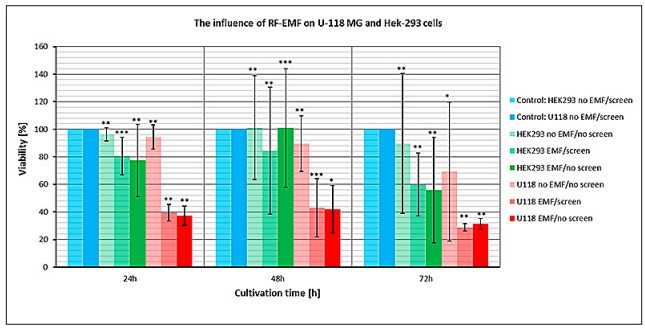



**Figure 8.** Changes in viability of U-118 MG and Hek-293 cells cultivated with and without RF-EMF and dielectric screen

In order to illustrate changes in the morphology and number of cells, photos of cells exposed to the electromagnetic field and control samples were taken. The analysis of U118 and Hek cells morphology under the influence of the electromagnetic field did not show any changes in relation to the control. Morphology was normal for this type of cells. The microscopic image clearly showed the influence of the electromagnetic field on the decrease in the number of cells. The greatest decrease occurred after 72 hours of incubation. Morphology of U-118 MG cells and changes those number cultivated with and without RF-EMF shown in Figures 9 and 10.

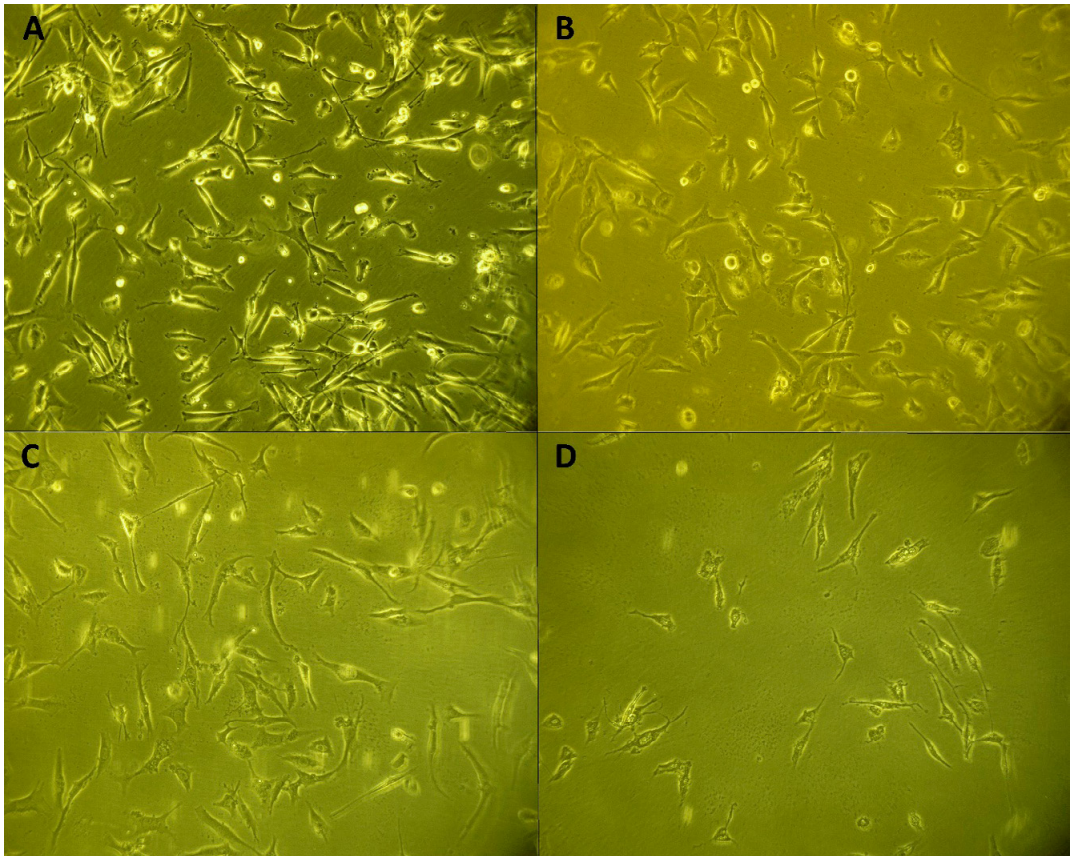



**Figure 9.** Microscopic image of U-118 MG cells cultivated in the electromagnetic field. A – cells 1h after the start of experiment; B – cells after 24h; C – cells after 48h; D – cells at the end of experiment (after 72h cultivation in the electromagnetic field).

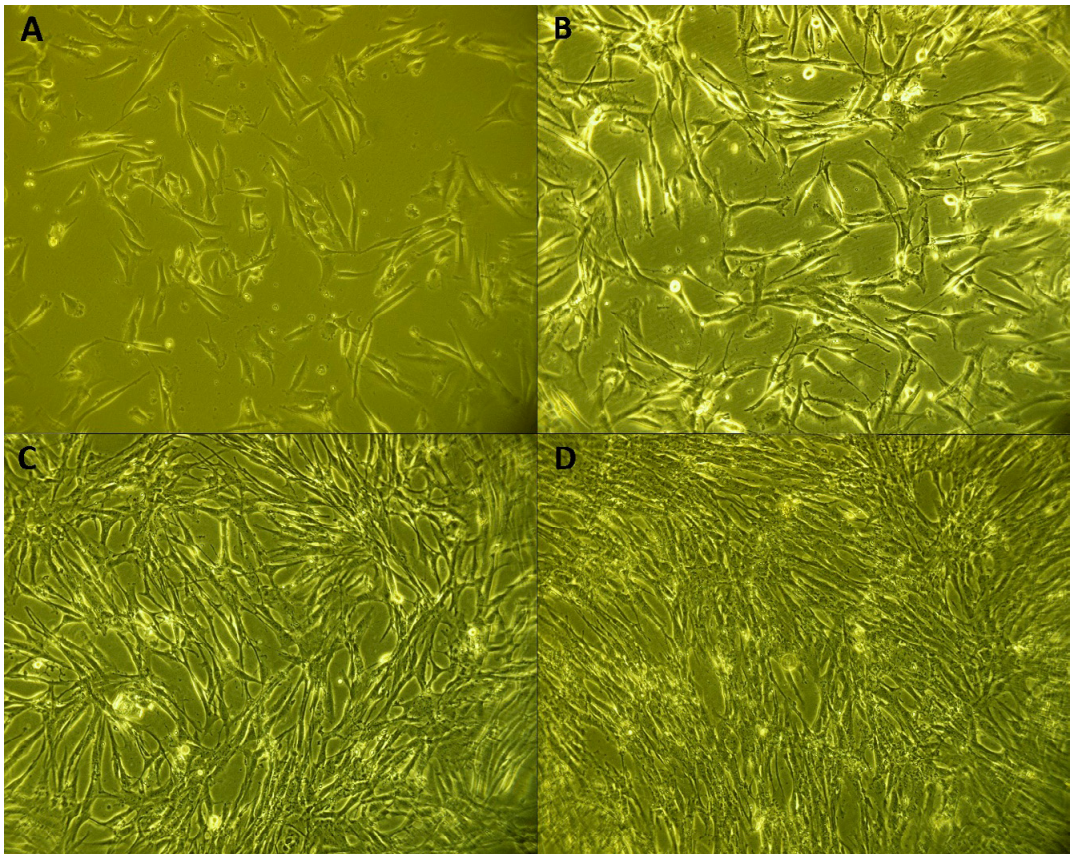



**Figure 10.** Microscopic image of U-118 MG cells cultivated without the electromagnetic field (control group). A – cells 1h after the start of experiment; B – cells after 24h; C – cells after 48h; D – cells at the end of experiment (after 72h of cultivation).

## DISCUSSION

Glioblastoma, the most common malignant tumour of the brain in adults, and unfortunately, increasingly often in children, is usually rapidly fatal. It is estimated that about 5 out of 100 000 people suffer from it annually. The average survival time of a patient diagnosed with glioblastoma, using current treatment standards, is just over 15 months from diagnosis, and median survival is estimated at 11 -12 months [33, 34]. Research shows that only 3 – 8% of patients live longer than three years [35]. Such a survival period, equal to and greater than 2.5 – 3 years from the diagnosis, is called long-term survival (LTS) [36, 37, 38].

Despite the progress made in recent years in the field of oncology, results of the treatment of malignant brain tumours remain unsatisfactory. The current standard of care for newly-diagnosed glioblastoma is surgical resection to the extent feasible, followed by adjuvant radiotherapy and chemotherapy. Unfortunately, due to the infiltrating nature of tumour growth and the location of glioblastoma in areas of the brain that are considered inoperable (due to their importance, for example, for speech understanding or conscious body movements), radical surgical treatment carried out in many cases without damaging these key brain areas is not possible. Generally, although patients may often be provided with the most promising prospects when the tumour is removed by surger, most patients tend to survive for a maximum five years after surgery [39]. The death of patients is mainly caused by tumour recurrence, and metastasis as a result of the remaining cancer cells which tend to be difficult to remove entirely during the surgery [40, 41, 42]. Unfortunately, patients are often unable to complete the prescribed chemotherapy cycles due to its adverse impact on their overall health. In view of the above, there is a considerable unmet need for an entirely different therapeutic approach, which may ensure better outcome and less toxicity.

Literature data indicate that electromagnetic radiation does not always have a negative effect on humans, including the development of cancer. It turns out that on the contrary, it can have a destructive effect on cancer cells. One generalized effect of the EMF exposure may be the morphological changes of the cancer cells as demonstrated by [43]. This was not confirmed in research by the authors of the current article. Morphology of the U-118 MG cancer cells was normal for this type of cells. It has been shown that electromagnetic fields (EMF) produce anti-cancer effects *in vitro*, affecting cell metabolism [44, 45]. The anti-cancer effect of the electromagnetic field was also found in the authors' *in vitro* studies who found that the RF electromagnetic field with the frequency 2.4 GHz affects negatively on metabolic activity of glioblastoma cells. It was found that this electromagnetic field had a significant influence on the decrease viability of glioblastoma U-118 MG and Hek-293 cells, but the effect on cancer cells was significantly stronger than on normal cells.

Markov and Vianale [46, 47] indicate that there are many factors affecting the biological responses, including amplitude, frequency and duration of exposure to the applied EMF, as well as the biological systems, e.g. cells, tissues and organisms. Akbarnejad et al. [44] suggest that the metabolic activity of brain glioblastoma cells depends on the frequency and amplitude of the applied electromagnetic field. They identified the impact of extremely low-frequency pulsed electromagnetic fields (ELF-PEMFs) applied at various frequencies and amplitudes in terms of its effect on cell cycle, apoptosis and viability of GBM cell line (U87) investigated *in vitro*. Analyses investigated morphological traits, cell viability and gene expression of proteins engaged in cell cycle regulation (Cyclin-D1 and P53) and apoptosis (Caspase-3). After 24h, the cell viability and Cyclin-D1 expression increased after exposure to 50 Hz, 100 G (30%, 45%), whereas they decreased at the exposure 100 Hz, 100 G (29%, 31%) and 10 Hz, 50 G (21%, 34%), whereas P53 and Caspase-3 were elevated only after exposure of cell line to 100 Hz, 100 G. According to the authors, the fact that some of the ELF-PEMFs frequencies and amplitudes arrest U87 cells growth could open the way to developing novel therapeutic approaches. Currently, high hopes are attached to the use of an electromagnetic field in the treatment of brain glioma. Some research results are very promising and indicate that therapeutic use of an electromagnetic field with the appropriate frequency and amplitude may become a new, effective and safe method for treating brain gliomas.

Lately tumour treating fields (TTFs), which is an antimitotic therapy using alternating electrical fields in order to disrupt the ability of cancer cells to divide, seems to be a novel promising strategy for the treatment of brain tumours aiming at providing an improved survival rate for many patients. The TTFs act upon specific highly charged proteins that are crucial for cell division; as a result, they are capable of slowing down tumour growth and the ability of the tumour to spread [48, 49]. In the treatment of brain gliomas a promising alternative approach uses an implantable ultrasound-powered tumour treating device (UP-TTD), which electromagnetically disrupts the rapid division of cancer cells, at the same time causing no adverse effects on normal neurons; consequently, it may safely inhibit the recurrence of brain cancer. Yang et al., in their i*n vitro* and *in vivo* experiments on tumour-bearing rats [45], showed a significant therapeutic effect of the UP-TTD, consisting in a ~58% inhibition of growth rate in the case of clinical tumour cells, and ~78% reduction in the area affected by cancer.

A recently approved treatment which applies electromagnetic fields alternating at 200 kHz, termed Optune™ therapy, is now available for recurrent *glioblastoma* multiform GBM, both as a monotherapy and used in combination with temozolomide for newly-diagnosed GBM [49]. This approach is also being tested in clinical trials to treat other cancers. It is hypothesised that the mechanism of its action includes disruption of tubulin dimers, mitotic spindles, and cell division as a result of electric field-induced dipole alignment and dielectrophoresis [50]. A modest effect of this procedure was also reported for the cell survival, increasing the median overall survival by 0.6 months in the case of recurrent GBM, whereas in recently-diagnosed GBM it was by 31% [49]. It should be stressed here that even this seemingly modest effect is considered significantly encouraging by patients.

Finally, it should be mentioned that before the start of the research for the current study, it turned out that the cell culture incubator connected to the electrical network generates strong electromagnetic radiation, and the power density was 0,35 mW/m$^2$. It was necessary to protect the tested samples in the control combination with a special dielectric screen, based on the ADR technology. This screen effectively absorbed the electromagnetic field emitted by the incubator,

which protected against the influence of an additional factor on cell metabolism. This research clearly showed the influence of radiation on the metabolic activity of the tested cell lines. Thus, in research conducted on living organisms inside incubators, it is necessary to protect the samples against harmful electromagnetic radiation generated by this device, otherwise the obtained results will be unreliable. For example, when examining the effect of drugs on the development of cancer cells, it will not be known until the end whether the electromagnetic field or the tested factor influenced the cell metabolism.

## CONCLUSIONS

The presented results indicate that 1) using a *radiofrequency* electromagnetic field (2.4 GHz) has a clearly negative effect on the metabolic activity of glioblastoma cells; 2) the RF electromagnetic field (RF-EMF) exposure caused a significant decrease viability of glioblastoma cells line U-118 MG; 3) RF-EMF has much less impact on reducing the viability of normal cells (Hek -293) than cancer cells.

In research conducted inside cell culture incubators, it is necessary to protect the samples against the influence of electromagnetic radiation generated by this device on cell metabolism. The dielectric screen constructed by ADR Technology used in the investigations effectively protects against the harmful effects of the electromagnetic field generated by the cell culture incubator.

## DECLARATION BY THE AUTHORS

The authors declare that they have no competing interests. All authors concurred with the submission and have seen a draft copy of the manuscript and agree with its publication. The manuscript has not been submitted for publication in any other journal.

## REFERENCES

1. GSMA Mobile Economy. 2018. https://www.gsma.com/mobileeconomy/wp-content/uploads/2018/05/The-Mobile-Economy-2018.pdf
2. Choi HD, Kim N. 5G jeonjapawa inche yeonghyang. In: Proceedings of the Korea Electromagnetic Engineering Soc. 2018;29:26–30.
3. Levitt BB, Lai H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. Environ Rev. 2011;19:495–495.
4. Woelders H, de Wit A, Lourens A, et al. Study of potential health effects of electromagnetic fields of telephony and wi-fi, using chicken embryo development as animal model. Bioelectromagnetics. 2017;38:186–203.
5. Sagar S, Adem SM, Struchen B, et al. Comparison of radiofrequency electromagnetic field exposure levels in different everyday microenvironments in an international context. Environ Int. 2018;114:297–306.
6. World Health Organization (WHO). Electromagnetic fields and public health: mobile phones. http://www.who.int/mediacentre/factsheets/fs193/en/. 2017; Accessed 17 Jan 2017.
7. Hardell L, Mild KH, Påhlson A, et al. Ionizing radiation, cellular telephones and the risk for brain tumours. Eur J Cancer Prev. 2001;10:523–529.
8. Hardell L, Carlberg M, Mild KH. Case–control study on cellular and cordless telephones and the risk for acoustic neuroma or meningioma in patients diagnosed 2000–2003. Neuroepidemiol. 2005a;25(3):120–128.
9. Hardell L, Sage C. Biological effect from electromagnetic field exposure and public exposure standards. Biomed Pharmacother. 2008;62(2):104–109.
10. Hardell L, Carlberg M, Söderqvist F, et al. Case-control study of the association between malignant brain tumours diagnosed between 2007 and 2009 and mobile and cordless phone use. Int J Oncol. 2013;43(6):1833–1845.
11. Prasad M, Kathuria P, Nair P, et al. Mobile phone use and risk of brain tumours: a systematic review of association between study quality, source of funding, and research outcomes. Neurol Sci. 2017;38(5):797–810.
12. INTERPHONE Study Group. Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. Int J Epidemiol. 2010;39:675–94.
13. Cardis E, Deltour I, Mann S, et al. Distribution of RF energy emitted by mobile phones in anatomical structures of the brain. Phys Med Biol. 2008;53:2771–2783.
14. Hardell L, Carlberg M, Mild KH. Pooled analysis of two case–control studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997–2003. Int Arch Occup Environ Health. 2006;79(8):630–639.
15. Wiart J, Hadjem A, Wong MF, et al. Analysis of RF exposure in the head tissues of children and adults. Phys Med Biol. 2008;53(13):3681–3695.
16. Gandhi OP, Kang G. Some present problems and a proposed experimental phantom for SAR compliance testing of cellular telephones at 835 and 1900 MHz. Phys Med Biol. 2002;47(9):1501–1518.
17. Sadetzki S, Chetrit A, Jarus-Hakak A, et al. Cellular phone use and risk of benign and malignant parotid gland tumors—a nationwide case–control study. Am J Epidemiol. 2008;167(4):457–467.
18. Hardell L, Eriksson M, Carlberg M, et al. Use of cellular or cordless telephones and the risk for non-Hodgkin's lymphoma. Int Arch Occup Environ Health. 2005b;8:625–632.
19. Linet MS, Taggart T, Severson RK, et al. Cellular telephones and non-Hodgkin lymphoma. Int J Cancer. 2006;119(10):2382–2388.
20. Hardell L, Carlberg M, Ohlson CG, et al. Use of cellular and cordless telephones and risk of testicular cancer. Int J Androl. 2007;30(2):115–122.
21. Lai H, Singh NP. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics. 1997;18:446–454.
22. Liu C, Duan W, Xu S, et al. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett. 2013;218:2–9.
23. Odaci E, Bas O, Kaplan S. ffects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain Res. 2008;1238:224–229.
24. Bas O, Odaci E, Kaplan S, et al. 900 MHz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat. Brain Res. 2009;1265:178–185.
25. Aldad TS, Gan G, Gao XB, et al. Fetal radiofrequency radiation exposure from 800–1900 mhz-rated cellular telephones affects neurodevelopment and behavior in mice. Sci Rep. 2012;2:312.
26. Salford LG, Brun AE, Eberhardt JL, et al. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. Environ Health Perspect. 2003;111(7):881–883.
27. Nittby H, Brun A, Eberhardt J, et al. Increased blood-brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. Pathophysiology. 2009;16(2–3):103–1012.
28. Maaroufi K, Save E, Poucet B, et al. Oxidative stress and prevention of the adaptive response to chronic iron overload in the brain of young adult rats exposed to a 150 kilohertz electromagnetic field. Neuroscience. 2011;186:39–47.
29. Dasdag S, Akdag MZ, Erdal ME, et al. Effects of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on microRNA expression in brain tissue. Int J Radiat Biol. 2015;91(7):555–561.
30. Kaplan S, Deniz OG, Önger ME, et al. Electromagnetic field and brain development. J Chem Neuroanat. 2016;75(B):52–61.
31. Wosiński S. The influence of the composition and manufacturing conditions of ceramic and polymer composites on the ability of shielding an alternating electric field, doctor thesis, 2010a. Poznan University of Technology.
32. Wosiński S. Solution for impregnation of materials shielding Low-Frequency Electric Field and the shielding material. International patent application. 2010b. WO/2010/093270.
33. Górski R, Nowak-Terpiłowska A, Śledziński P, Baranowski M, Wosiński S. Morphological and cytophysiological changes in selected lines of normal and cancer human cells under the influence of a radio-frequency electromagnetic field. Ann Agric Environ Med. 2021;28(1):163–171.
34. ESP8266EX manual.

35. Krex D, Klink B, Hartmann C, et al. Long-term survival with glioblastoma multiforme. Brain. 2007;130:2596–2606.
36. Pan W, Ferguson S, Lam S. Patient and treatment factors associated with survival among adult glioblastomas patients: a USA population-based study from 2000–2010. J Neurosci. 2015;22:1575–1581.
37. Amelot A, De Cremoux P, Quillien V. IDH-Mutation is a weak predictor of long-term survival in glioblastoma patients. PLoS One. 2015;10: e0130596.
38. Johnson D, Ma D, Buckner J, et al. Conditional probability of long-term survival in glioblastoma: a population-based analysis. Cancer. 2012;118:5608–5613.
39. Smoll N, Schaller K, Gautschi OJ. Long-term survival of patients with glioblastoma multiforme (GBM). J Clin Neurosci. 2013;20:670–675.
40. Gaist D, Hallas J, Friis S. et al. Statin use and survival following glioblastoma multiforme. Cancer Epidemiol. 2014;38:722–727.
41. Gittleman H, Boscia A, Ostrom QT, et al. Survivorship in adults with malignant brain and other central nervous system tumor from 2000–2014. Neuro Oncol. 2018;20:vii6–vii16.
42. Peters S, Bexelius C, Munk V, et al. The impact of brain metastasis on quality of life, resource utilization and survival in patients with non-small-cell lung cancer. Cancer Treat Rev. 2016;45:139–162.
43. Churilla TM, Chowdhury IH, Handorf E, et al. Comparison of local control of brain metastases with stereotactic radiosurgery vs surgical resection: A secondary analysis of a randomized clinical trial. JAMA Oncol. 2019;5:243–247.
44. Suh JH, Kotecha R, Chao ST, et al. Current approaches to the management of brain metastases. Nat Rev Clin Oncol. 2020;17:279–299.
45. Hasanzadeh H, Rezaie-Tavirani M, Seyyedi SS, et al. Effect of ELF-EMF Exposure on Human Neuroblastoma Cell Line: a Proteomics Analysis. Nat Rev Clin Oncol. 2014;7:22–27.
46. Akbarnejad Z, Eskandary H, Vergallo C, et al. Effects of extremely low-frequency pulsed electromagnetic fields (ELF-PEMFs) on glioblastoma cells (U87). Electromagn Biol Med. 2017;36(3): 238–247.
47. Yang Y, Hu X, Liu Y, et al. An implantable ultrasound-powered device for the treatment of brain cancer using electromagnetic fields. Sci. Adv. 2022;8(29):eabm5023.
48. Markov MS Electromagnetic Fields in Biology and Medicine. Boca Raton: CRC Press, Taylor & Francis Group. 2015;476pp.
49. Vianale G, Reale M, Amerio P, et al. Extremely low frequency electromagnetic field enhances human keratinocyte cell growth and decreases proinflammatory chemokine production. Br J Dermatol. 2008;158:1189–1196.
50. Mrugala MM, Engelhard HH, Tran DD, et al. Clinical practice experience with NovoTTF-100A system for glioblastoma: The Patient Registry Dataset (PRiDe). Semin Oncol. 2014;41Suppl 6, S4–S13.
51. Stupp R, Taillibert S, Kanner A, et al. Effect of tumortreating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: A randomized clinical trial. JAMA. 2017;318:2306–2316.
52. Tuszynski JA, Wenger C, Friesen DE, et al. An Overview of Sub-Cellular Mechanisms Involved in the Action of TTFields. Int J Environ Res Public Health. 2016;12;13(11):1128.