

Effects of 27.12 MHz short-waves on fibroblast cell culture and K-562 and ML-1 neoplastic cell lines

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ABSTRACT

Objectives: This study aimed to assess the effect of short-wave therapy (SWT) devices emitting radiofrequency (RF) waves on the proliferation rates of fibroblasts and neoplastic cells.

Patients and methods: In this experimental study, fibroblasts cultured from sternal mesenchymal cells of a bypass surgery patient were enriched using stem cell techniques between January 2004 and February 2004. The K-562 and ML-1 neoplastic cell lines were prepared for analysis. Fibroblasts and neoplastic cell lines were exposed to 27.12 MHz short-waves at different energy levels. Continuous short-wave (CSW) was applied at 200 W power, and pulsed short-wave (PSW) was applied at three different mean powers: 1.6 W (PSW-1), 14.9 W (PSW-2), and 54 W (PSW-3). Fibroblast colonies were counted using inverted microscopy, and neoplastic cell proliferation rates were measured using enzyme-linked immunosorbent assay. All short-wave-exposed cells were compared to the controls with no exposure.

Results: Short-waves increased the number of fibroblast colonies three- to four-fold across all power levels (1.6 W, 14.9 W, 54 W, and 200 W). They significantly increased K-562 cell proliferation only at 1.6 W and 54 W power levels ($p=0.044$ and $p=0.004$, respectively). In contrast, there was no significant increase in ML-1 cell proliferation at any power level tested ($p>0.05$).

Conclusion: This study found that short-waves can boost fibroblast proliferation, potentially aiding tendon healing. However, it also had unpredictable proliferative effects on K-562 cells, as an inconsistent correlation with energy levels was observed. The ML-1 cells were not affected by short-waves, suggesting variability in tumor biology. These findings emphasize the need for precise dosing and personalized treatment strategies when using SWT devices.

Keywords: Cell proliferation, fibroblast cell, radiofrequency, short-wave therapy.

Short-wave therapy (SWT) devices emitting short-waves at frequencies 13.56 MHz or 27.12 MHz have been used in therapeutic applications. They belong to the radiofrequency (RF) subgroup of electromagnetic waves with frequencies ranging from 30 kHz to 300 GHz. This subgroup also includes technologies, such as mobile phones, radio broadcasting, and microwave ovens.

Short-wave therapy devices generate heat in body parts when operated in continuous mode owing to the rapid movement of ions and the rotation of dipolar molecules. This heat accelerates cellular metabolism, oxygen consumption, and energy expenditure

and facilitates repair processes.^[1] However, pulsed applications have minimal thermal effects in tissues as heat dissipation occurs between pulses. Although nonthermal, there is evidence that pulsed short-wave (PSW) changes ion channels and receptors in cell membranes.^[2] In a systematic review of 16 studies, PSW was found to have beneficial effects on pain, stiffness, and physical function in osteoarthritis.^[3] According to a report by the Food and Drug Administration on short-wave diathermy, continuous short-wave (CSW) is indicated for musculoskeletal pain, muscle spasms, and joint contractures, whereas PSW is recommended for postoperative pain and soft tissue edema.^[4]

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Received: January 16, 2024 **Accepted:** June 10, 2024 **Published online:** July 26, 2024

Cite this article as: Yılmaz Muluk S, Özsan GH, Öncel S, Ateş H. Effects of 27.12 MHz short-waves on fibroblast cell culture and K-562 and ML-1 neoplastic cell lines. Turk J Phys Med Rehab 2025;71(x):i-ix. doi: 10.5606/tftrd.2024.14635.



Although some studies have suggested a potential link between RF waves and carcinogenicity, the International Agency for Research on Cancer does not classify RF waves as human carcinogens. This decision stems from the recognition of potential biases and limitations affecting the findings of such studies.^[5]

Despite the long history of the clinical use of SWT devices, there are significant knowledge gaps regarding their effects on cellular proliferation. Whether SWT devices stimulate human fibroblasts and neoplastic cell lines remains largely unknown. Therefore, this study is significant as it fills this gap.

This study aimed to investigate the impact of repeated 27.12 MHz short-wave exposure on normal human fibroblast proliferation and the effect of a single 27.12 MHz short-wave dose on K-562 and ML-1 neoplastic cell proliferation. In line with these objectives, we hypothesized that repeated 27.12 MHz short-wave exposure would enhance normal human fibroblast proliferation, potentially aiding tendon healing. Simultaneously, we expected variable effects on the neoplastic cell lines depending on the power of the waves.

PATIENTS AND METHODS

This study was based on the original dissertation conducted by the first author and was carried out in the adult hematology-oncology laboratory and the SWT room of the physical therapy unit of Dokuz Eylül University Faculty of Medicine between January 2004 and February 2004. Cell culture conditions, cell line processing procedures, transportation techniques, and short-wave exposure practices were standardized. The experimental conditions were meticulously designed to closely replicate physiological conditions. The incubator was maintained at 37°C, and the SWT room temperature was set to 37°C during applications to simulate human body conditions, ensuring the relevance of the observed cellular responses to physiological norms.

During each application session, all the cell culture flasks and cell line tubes were carefully positioned on a wooden table between the plates of the SWT device. This distance was accurately measured as 3 cm from each of the two plates, running parallel to them, and was standardized for each application. Control flasks and tubes, which were intended to remain unexposed to short-waves, were removed from the incubator and transported to the area; however, they were positioned more than 3 m away from the SWT device.

A sterile environment for handling and storing the flasks and tubes was maintained throughout the study, with the flasks and tubes sterilized after each application before being placed inside the incubator.

Procedure of mesenchymal cell culture

A 65-year-old female patient who was scheduled for open bypass surgery and had no disease other than coronary artery disease was chosen. During surgery, the surgeon took a 15-mL sample of bone marrow cells from the sternum into a heparinized injector. The specimen was then immediately transported to the laboratory. The mesenchymal stem cell enrichment method was used to obtain fibroblast colonies.

The sample was diluted in a one-to-one ratio by adding phosphate-buffered saline containing 2% fetal bovine serum (Biological Industries, Beit Haemek, Israel). The diluted sample was placed in conical bottom centrifuge tubes and layered on Histopaque-1077 (Sigma Diagnostics Inc., St. Louis, Missouri, USA), which can isolate bone marrow mononuclear cells (MNCs). The mixture was then centrifuged at 400× g at room temperature for 20 min. After centrifugation, the MNC layer that formed over the interphase layer was removed. Phosphate-buffered saline containing 2% fetal bovine serum was added, and the cells were centrifuged again at 250× g at room temperature for 10 min. The upper portion of the liquid was discarded, and the remaining sinking MNC pellet was resuspended in the complete medium. The complete medium included one unit of mesenchymal stem cell-stimulating supplement (StemCell Technologies, Vancouver, Canada) and nine units of basic medium for human mesenchymal stem cells (Mesencult; StemCell Technologies, Vancouver, Canada). Cell viability was tested with trypan blue (Sigma Diagnostics Inc., St. Louis, Missouri, USA) before seeding and was found to be >90%. Cells were then counted with the Coulter STKS device (Beckman Coulter, Inc., Brea, California, USA) and randomly seeded into five 25 cm² T-flasks (Corning Inc., New York, USA) such that there were 1×10⁶ MNC in 10 mL of complete medium in each flask. Penicillin (100 µg/mL), streptomycin (100 µg/mL), and amphotericin B (4 µg/mL) were added to environments. The flasks were incubated for 14 days in a humid environment containing 5% carbon dioxide (CO₂) at 37°C.^[6]

Exposure of fibroblast cells

Although the duration and treatment frequency of SWT applications have not been precisely

Flask/tube radiation	Labeling	Power/mean power for PSW
No radiation	Control	–
Continuous radiation	CSW	200 W
Pulsed radiation	PSW-1	1.6 W (PP 200 W, PRF 20 Hz)
Pulsed radiation	PSW-2	14.9 W (PP 600 W, PRF 62 Hz)
Pulsed radiation	PSW-3	54 W (PP 900 W, PRF 150 Hz)

PSW: Pulsed short-wave; PP: Pulse power/peak power; PRF: Pulse repetition frequency; Pulse durations are 400 μ sec.

reported, the common clinical use is 15 to 30 min for 10 to 20 sessions.^[3] We planned a 15-min application twice daily for five days. Starting from the fifth day of incubation, waves were applied to the four flasks at predetermined doses. Continuous short-wave was applied at 200 W power. Pulsed short-wave was applied at three different mean powers: 1.6 W (PSW-1), 14.9 W (PSW-2), and 54 W (PSW-3; Table 1).

The flasks were placed between the electrodes of the Curapuls 419 device (Enraf Nonius, Rotterdam, the Netherlands). The control flask was kept outside the incubator for the same duration but was not exposed to any waves. The flasks were returned to the incubator immediately after application and maintained there until the end of the incubation period, that is, until the 14th day.

Analysis of fibroblast colonies

On the 14th day, flasks were removed from the incubator. The cells were stained with Giemsa, and fibroblast colonies were examined under inverted and light microscopy. Figure 1 shows the fibroblast colonies observed under a light microscope at two different magnifications. Groups containing an average of 50 or more cells were considered colonies. Colonies ranging from 1 to 8 mm in diameter were visible macroscopically at the base of the flasks. All fibroblast colonies were counted in each flask both microscopically and with the naked eye against a grid background by a hematologist who was blinded to the doses given.

Processing of neoplastic cells

The K-562 and ML-1 neoplastic cell lines (European Collection of Cell Cultures, Salisbury, UK) were obtained from distributor sources for research purposes. The K-562 cell line includes neoplastic cells derived and amplified in 1970 from the pleural effusion of a 53-year-old patient with chronic myeloid leukemia (CML) during the terminal blast

crisis. Cells have lymphoblast morphology. Recent studies have shown that K-562 cells are multipotent hematopoietic neoplastic cells that can spontaneously differentiate into the progenitors of erythrocytes, granulocytes, and monocytes.^[7] The ML-1 cell line was isolated in 1978 from the peripheral blood of a 24-year-old patient with acute myeloid leukemia

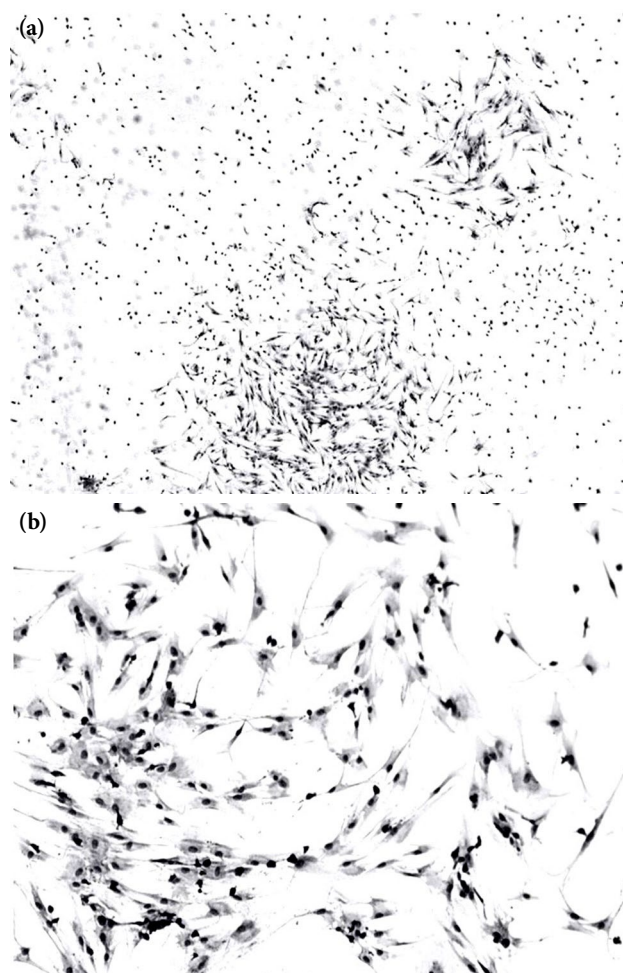


Figure 1. Fibroblast colonies under a light microscope at two different magnifications: (a) $\times 40$, (b) $\times 100$.

(AML). Cell morphology was lymphoid-like.^[8] The K-562 and ML-1 cell lines were stored at 170°C using dimethyl sulfoxide as a cryoprotectant. To begin the experiment, they were dissolved in a water bath at 37°C and placed in sterile conical centrifuge tubes. An RPMI 1640 medium (Biological Industries, Beit Haemek, Israel) was then added to the cells. The samples were centrifuged at 250× g at 4°C for 5 min. After centrifugation, the upper portion of the supernatants were discarded. The RPMI 1640 medium was added to the cell pellets remaining at the bottom of the tubes and centrifuged again at 250× g and 4°C for 5 min. Washing was performed twice at 4°C. The samples were then placed in T-25 cm² flasks and kept in a humid environment containing 5% CO₂ at 37°C for 48 h.^[9]

Exposure of neoplastic cells

After 48 h, neoplastic cells were randomly placed in sterile conical tubes. Five tubes, each containing the same number of cells, were prepared for each cell line. On the same day, 15 min of 27.12 MHz short-waves were applied to four tubes at predetermined doses, analogous to the doses applied to the fibroblast flasks (Table 1). The same SWT device was used, and the tubes were placed between the electrodes of the device. Control tubes were kept outside the incubator for the same duration and were not irradiated. As neoplastic cells have a shorter doubling time, they were exposed to short-waves only once, and the results were planned to be observed earlier.

The tubes were brought back to the laboratory after application. First, the viability of the cells was

tested using trypan blue and found to be >90%. The cells were then randomly seeded into a flat-bottom culture dish containing 96 wells such that each well contained 1×10⁶ cells. The dish was then placed in the incubator at 37°C in a humid medium containing 5% CO₂ and maintained there for the next three days.^[10] The arrangement of the 96-well culture dish is shown in Figure 2.

Analysis of neoplastic cells

On the fourth day, the culture dish was removed from the incubator. To assess cell proliferation rates, we used the XTT Cell Proliferation Kit II (Roche, Basel, Switzerland), which utilizes a colorimetric indicator. Specifically, 50 µL of XTT reagent was added to each well of the culture dish, including four empty wells without cell cultivation. This step was essential for the spectrophotometric quantification of cell proliferation.

The culture dish was then incubated for an additional 4 h at 37°C in a humid environment containing 5% CO₂, as described by Huyck et al.^[10] After a 4-h incubation period, we conducted colorimetric analysis using enzyme-linked immunosorbent assay at a wavelength of 490 nm to calculate cell proliferation rates.

Statistical analysis

Data analysis was performed using GraphPad Prism version 10.0.2 (GraphPad Software Inc., San Diego, CA, USA). Owing to the nature of this type of experiment, each group contained a limited number of samples. For this reason, we used percentage calculations for fibroblast colony counts and used Dunn's test (nonparametric, used after the Kruskal-Wallis test) and Dunnett's test [parametric, used in one-way ANOVA (analysis of variance) with a control group] for neoplastic cell proliferation rates. The data for the exposed fibroblast cells were expressed as percentages relative to the unexposed control set at 100%.

To determine the actual proliferation values of the short-wave-exposed neoplastic cells, we used the mean optical density values of the empty cells. The absorbance values in the empty wells were 0.172, 0.163, 0.152, and 0.139, with a mean of 0.157±0.012. This mean value was subtracted from the initial optical density values of the tested wells. The resulting actual cell proliferation values for K562 and ML-1 cells are presented in Table 2. We then compared the control group of neoplastic cell lines to the short-wave-exposed groups.

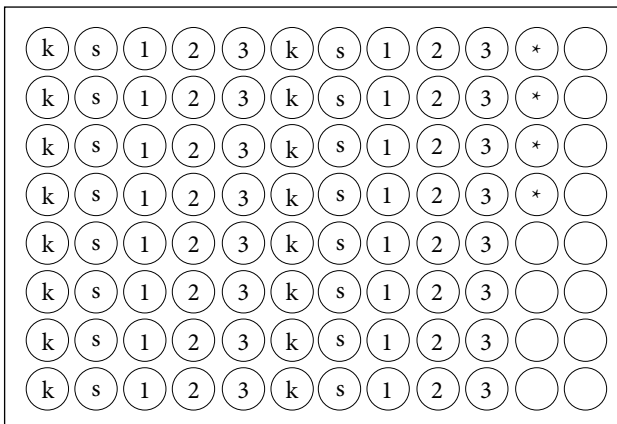


Figure 2. Arrangement of the 96-well culture dish.

k: Control cells; s: CSW-applied cells; 1: PSW-1-applied cells; 2: PSW-2-applied cells; 3: PSW-3-applied cells. Wells marked with * have no cells seeded; Left five columns: K-562, next five columns: ML-1; CSW: Continuous short-wave; PSW: Pulsed short-wave.

Despite the small sample size, one-way ANOVA was employed to examine the differences in cell proliferation among the groups. This choice was driven by the appropriateness of the test for comparing means across multiple groups, which aligned directly with our research objectives. Normality tests were also conducted to assess the normal distribution assumption of one-way ANOVA. Although these tests may not reliably detect deviations from normality, the results of the One-way ANOVA were presented for full transparency.

Subsequently, as the assumptions regarding data distribution were not met for the K-562 cell values, alternative statistical methods, such as nonparametric tests, were used to strengthen the analysis. The Kruskal-Wallis test and Dunn's multiple comparisons test, which were intentional, were more robust to small sample sizes and did not assume a normal distribution of data. This approach complemented the parametric test we used, offering a comprehensive view of the data. This dual approach enriched the depth and robustness of the analysis. As the assumptions about data distribution were met for the ML-1 cell values, Dunnett's multiple comparisons test was used for the analysis. The level of statistical significance was set at $p < 0.05$.

RESULTS

For fibroblasts, colony counts in the control, CSW, PSW-1, PSW-2, and PSW-3 flasks were 25, 72, 100, 81, and 95, respectively. The colony increase (three- to four-fold) was not directly proportional to the mean power. The increase in colony counts also did not correlate directly with mean power, peak power, frequency, or mode of energy delivery, whether

continuous or pulsed. Compared to the control (set at 100%), the increases were 188% (CSW), 300% (PSW-1), 224% (PSW-2), and 280% (PSW-3; Table 3).

For K-562 neoplastic cells, the control group was compared with the irradiated groups. Initially, differences in cell proliferation means among the groups were examined (one-way ANOVA). However, normality was not met for the data (p -values for normality tests: D'Agostino-Pearson=0.007, Anderson-Darling=0.004, Shapiro-Wilk=0.005, and Kolmogorov-Smirnov=0.048). Nonetheless, owing to the robustness of the method to deviations from normality, we proceeded with the analysis. There was a significant difference between the mean values of the groups ($F=2.933$, $p=0.034$), indicating that at least one group differed significantly from the others in terms of cell proliferation. Given the departure from normality, a nonparametric test was also conducted to evaluate whether significant differences in medians existed among the groups. There was a statistically significant difference between the groups ($p=0.002$, Kruskal-Wallis test). This further supported the findings of the one-way ANOVA. Subsequently, significant differences in the median ranks were observed between the control group and both PSW-1 ($p=0.044$) and PSW-3 ($p=0.004$). However, comparisons between the control group and CSW ($p=0.307$), as well as PSW-2 ($p=0.990$), showed no significant differences (Dunn's multiple comparisons test; Table 4).

The combined results of these tests indicated significant differences in cell proliferation patterns between the control and irradiated K-562 cell groups. These findings suggest that specific radiation protocols, such as PSW-1 and PSW-3, increased K-562 cell proliferation compared to the control group.

TABLE 2
Actual cell proliferation values of neoplastic cells

Wells	Control	CSW	PSW-1	PSW-2	PSW-3	Control	CSW	PSW-1	PSW-2	PSW-3
1	0.237	0.194	0.146	0.157	0.157	0.338	0.327	0.345	0.372	0.391
2	0.175	0.183	0.182	0.141	0.208	0.437	0.397	0.427	0.403	0.442
3	0.137	0.179	0.203	0.169	0.190	0.433	0.469	0.476	0.514	0.404
4	0.156	0.207	0.184	0.166	0.203	0.372	0.388	0.379	0.418	0.400
5	0.134	0.194	0.206	0.174	0.203	0.414	0.471	0.413	0.434	0.449
6	0.139	0.185	0.202	0.240	0.226	0.424	0.435	0.429	0.447	0.404
7	0.156	0.179	0.235	0.171	0.260	0.416	0.473	0.408	0.392	0.395
8	0.179	0.174	0.184	0.134	0.190	0.494	0.438	0.358	0.538	0.538

CSW: Continuous short-wave; PSW: Pulsed short-wave. Cell proliferation rates are determined through colorimetric analysis using enzyme-linked immunosorbent assay at a wavelength of 490 nm. The actual values are obtained by subtracting the mean optical density values of empty cells from the data.

TABLE 3
Comparison of radiated fibroblast cells to the control

Groups	Colony count	Increase compared to control	Percentage increase
Control	25	–	100
CSW	72	47	188
PSW-1	100	75	300
PSW-2	81	56	224
PSW-3	95	70	280

CSW: Continuous short-wave; PSW: Pulsed short-wave. The colony number in the control flask was set at 100%, and the colony numbers in the short-wave-exposed flasks were compared to the control.

TABLE 4
Comparative analysis of neoplastic cells exposed to 27.12 MHz short-wave radiation

Cell type	Comparison	p-value	95% CI	Conclusion
K-562	Control vs. CSW	0.307	–0.058 - 0.013	NS
K-562	Control vs. PSW-1	0.044	–0.064 - 0.007	S
K-562	Control vs. PSW-2	0.990	–0.040 - 0.031	NS
K-562	Control vs. PSW-3	0.004	–0.076 - –0.005	S
ML-1	Control vs. CSW	0.990	–0.073 - 0.055	NS
ML-1	Control vs. PSW-1	0.972	–0.052 - 0.075	NS
ML-1	Control vs. PSW-2	0.745	–0.088 - 0.040	NS
ML-1	Control vs. PSW-3	0.970	–0.076 - 0.052	NS

CSW: Continuous short-wave; PSW: Pulsed short-wave; CI: Confidence interval; NS: No significant difference; S: Significant difference in cell proliferation rates. Dunn's multiple comparisons test was used for K-562 cells where assumptions were not met, while Dunnett's multiple comparisons test was applied to ML-1 cells, fulfilling the assumptions.

The combined results of these tests indicated significant differences in cell proliferation patterns between the control and irradiated K-562 cell groups. These findings suggested that specific radiation protocols, such as 1.6 W (PP 200 W, PRF 20 Hz) and 54 W (PP 900 W, PRF 150 Hz), increased K-562 cell proliferation.

For ML-1 neoplastic cells, the control group was compared with the irradiated groups. The analysis conducted to evaluate potential differences in cell proliferation between experimental groups indicated no significant differences between the groups ($F=0.5649$, $p=0.690$; one-way ANOVA). The assumption of normality was met for the data (p -values for normality tests: D'Agostino-Pearson=0.607, Anderson-Darling=0.434, Shapiro-Wilk=0.635, and Kolmogorov-Smirnov=0.1000). Furthermore, the assumption of homogeneity of variance was supported by the nonsignificant results of the Brown-Forsythe ($p=0.936$) and Bartlett's tests ($p=0.943$). Given the absence of significant differences, multiple comparisons were

employed to compare each irradiated group with the control group. There were no statistically significant differences: CSW ($p=0.990$), PSW-1 ($p=0.972$), PSW-2 ($p=0.745$), and PSW-3 ($p=0.970$; Dunnett's multiple comparisons test; Table 4).

These tests revealed no significant differences in the proliferation of ML-1 cells between the control and irradiated groups, suggesting that short-wave radiation did not alter the proliferation of ML-1 cells.

DISCUSSION

Radiofrequency waves, characterized by their nonionizing nature, are generally considered safer than ionizing radiation forms such as gamma rays and X-rays. These waves are widespread in our environment as they are emitted from sources such as TV, radio broadcasts, mobile phones, and microwave ovens.^[11] Short-wave therapy devices, which also emit RF waves, are often considered safe but still require a thorough investigation of their potential risks.

This study investigated the effect of 27.12 MHz short-waves on normal human fibroblasts and neoplastic K-562 and ML-1 cell lines and is the first study to our knowledge to investigate these effects on K-562 and ML-1 cells.

In the first part of this study, fibroblast colonies increased at all power levels. Similarly, an earlier study on the impact of short-waves on fibroblasts and chondrocytes revealed that PSW therapy stimulates cell proliferation.^[12] Additionally, this proliferation was found to be directly proportional to the energy transferred, identifying an optimal mean power of 13.8 W for cell proliferation. This latter finding contrasts with our results, in which the increase in proliferation was not directly proportional to the mean power. The increases we observed did not also correlate with the peak power or frequency of short-waves.

A separate study on the effects of a 960 MHz RF field, commonly used in the GSM (Global System for Mobile Communications), on human epithelial amnion cells was conducted at two distinct temperatures: 39°C and 35°C.^[13] A notable increase in cell proliferation was observed at both temperatures. This suggests that the biological influence of RF radiation extends beyond thermal effects, highlighting the need for further investigation of nonthermal effects. In our experiment, the temperature was set to 37°C to reflect the typical human body conditions.

In the second part of the study, significant increases were observed in K-562 cell proliferation in specific settings. However, no significant changes were present in ML-1 cell proliferation under any of the short-wave exposure settings. These outcomes suggest that 27.12 MHz short-waves at mean powers of 1.6 W and 54 W may promote tumor progression in K-562 cells but not in ML-1 cells. It is important to note that the increase in K-562 cell proliferation was not directly proportional to the mean power, the peak power, or the frequency of the short-waves.

Literature on the potential carcinogenic effects of RF waves is complex. Cardis et al.^[14] highlighted a possible correlation between the long-term use of mobile phones and an elevated risk of glioma. Another study showed that exposure to RF radiation may cause DNA strand breaks, indicating potential genetic damage.^[15] Additionally, specific *in situ* experiments have demonstrated genotoxic effects in Tradescantia plants exposed to short-wave electromagnetic fields.^[16] In our study, pulsed radiation at 1.6 W and 54 W significantly enhanced

proliferation in CML-derived cells, while neither 14.9 W pulsed nor 200 W continuous radiation had a similar effect.

In contrast, a review conducted by Jauchem^[17] reported minimal or no substantial evidence linking low-level microwave exposure with adverse health effects. This perspective was further supported in another one of Jauchem's^[18] reviews, which focused on both occupational and residential exposure to RF energy, including microwaves, again finding little evidence of harmful health impacts. This view is supported by another study that was unable to establish definitive connections between RF radiation and brain or infant cancers.^[19] Consistent with these reports, in our study, none of the energy levels tested enhanced the proliferation of AML-derived cells.

Surprisingly, recent studies have highlighted the potential therapeutic benefits of RF waves, particularly in cancer treatment. Low-power millimeter waves have demonstrated selective inhibitory effects on tumor cell growth in various human neoplastic cell lines.^[20] Further research has revealed that specific frequencies of these waves can impede growth and alter the morphology of cells in human melanoma and breast cancer lines.^[21,22] Besides, RF waves are emerging as a nonsurgical alternative for certain thyroid cancer treatments.^[23] They have also been found to affect cell phenotype and mitochondrial function in pancreatic cancer cell lines.^[24,25] Of particular interest, a study demonstrated that very low levels of 27.12 MHz RF waves, specifically at specific absorption rates ranging from 0.05 to 1.0 W/kg and with daily exposures of 6 h, effectively inhibited growth in hepatocellular cancer cell lines without harming normal tissues. This suggests a potentially significant role of these waves in oncological treatments.^[26]

While some studies in the literature suggest possible carcinogenic effects of RF waves, others indicate minimal health risks and some even highlight potential therapeutic benefits for cancer treatment.

In our research, fibroblasts showed an unexpected nonlinear response with no increase in proliferation at higher mean power levels. This suggests that the optimal RF energy level for peak fibroblast proliferation may vary with power setting. Similarly, K-562 cells showed an unexpected response. The increase in mean power did not affect proliferation; however, PSWs significantly enhanced growth, whereas CSWs had no effect despite their thermal properties. This suggests that intermittent wave patterns may activate specific cellular mechanisms

not triggered by continuous radiation, and constant stress with continuous radiation might engage defensive responses such as DNA repair and cell cycle arrest, favoring cellular stability over proliferation. Another unexpected finding for the K-562 experiment was that among the pulsed exposure settings, the increase in cell proliferation occurred at mean power settings of 1.6 W and 54 W but not at 14.9 W. This variability underscores the complex impact of RF energy on cell behavior across different power settings.

Another finding of our study was that 27.12 MHz short-wave radiation, at the settings we used, significantly stimulated the proliferation of CML-derived cells but not of AML-derived cells. This variation may have stemmed from differences in tumor cell biology. Considering the contradictory findings in the literature and the results of our study, further research is needed to better understand the effects of RF on cell proliferation.

The primary limitation of our study was its small sample size, which is a common challenge in cell culture and cell line laboratory studies. This limitation could potentially affect the ability to detect real effects. Acknowledging this typical limitation in laboratory research, we aimed to enhance the reliability of our results by employing a combination of parametric and nonparametric tests for the evaluation of neoplastic cell lines. Another limitation was the variation in treatment frequencies. The fibroblast cultures underwent radiation exposure in ten separate sessions, while the neoplastic cells were subjected to a single short-wave exposure due to their shorter doubling times, which permitted earlier observation of the results. Owing to this divergent experimental design, direct quantitative comparisons between the two sets of experiments were unfeasible. As such, we presented the results of fibroblast cultures separately and limited our comparison to the two cell lines themselves, which is consistent with the original two-part design of the study. Despite these limitations, our study provides valuable insights and its significance lies in its contribution to a topic with limited available data. Future studies should consider expanding the sample size and incorporating various cell proliferation assays other than the XTT assay that we used, along with cell cycle analyses, apoptosis assays, and functional investigations. It is also essential to diversify the cell lines and energy levels of RF waves to ensure a comprehensive exploration

of this topic. Moreover, considering that CML patients often receive tyrosine kinase inhibitors such as imatinib indefinitely, which could influence cellular responses, future studies can be designed to include imatinib-added K-562 cells. This approach will examine how tyrosine kinase inhibition might modify the proliferative responses to SWT, aiding in understanding the interactions between RF therapy and ongoing pharmacological interventions.

In conclusion, this study demonstrated that SWT can significantly enhance fibroblast proliferation, potentially aiding tendon healing; however, careful consideration of its effects on neoplastic situations is crucial. The complex and sometimes contradictory nature of RF wave interactions with biological tissues underscores the importance of precise dosing and personalized treatment strategies for the use of SWT devices. The discovery of specific frequency ranges in SWT devices that can stimulate healthy cell growth while inhibiting cancer cell proliferation holds promise for future therapies.

Ethics Committee Approval: As our study focused exclusively on cell cultures and cell lines without human or animal involvement, ethics committee approval was not required by our institution's regulations. Nonetheless, the study strictly adhered to the principles of the Declaration of Helsinki.

Patient Consent for Publication: Written informed consent was obtained from the patient having bypass surgery.

Data Sharing Statement: Data supporting the findings of this study are available upon reasonable request. They are also present in the public repository ZENODO (doi: 10.5281/zenodo.8333479).

Author Contributions: Contributors idea, literature review, writing the article: S.Y.M.; Concept design control, analysis and/or interpretation: S.Y.M., G.H.Ö., S.Ö.; Supervision data collection and/or processing, critical review: S.Y.M., G.H.Ö., S.Ö., H.A.; References and fundings, materials: S.Y.M., G.H.Ö., H.A.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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