2004

The Effects of a One-Tesla Magnet on Human Fibroblast Cell Growth

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by

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A Thesis Submitted in Partial Fulfillment of
Requirements of the CSU Honors Program

for Honors in the degree of
Bachelor of Science
in
Biology,
College of Science,
Columbus State University

Thesis Advisor _____ Date 5 Nov 2004

Committee Member _____ Date 5 Nov. '04

Committee Member _____ Date 5 Nov 2004

CSU Honors Committee Member Barbara Hunt Date 11/5/04

Coordinator, Honors Program Barbara Hunt Date 11/5/04

Gbt 2-3-05

Abstract

The controversy regarding the ill effects of electromagnetic fields began in 1979 when Nancy Wertheimer and Ed Leeper claimed to have found a connection between childhood leukemia and power lines. In 1997, a group of researchers led by Martha Linet, M. D., attempted to provide evidence that there was no link between cancer and electromagnetic fields. The study showed that the risk of acute lymphoblastic leukemia did not escalate with increasing electromagnetic field levels in the children's homes. When compared to the Earth's static magnetic field of 0.5 Gauss, these fields were extremely small. Within the past decade, magnets have been used to treat various medical conditions including arthritis and migraines. Magnetotherapy is the term coined for this alternative approach to medicine which requires placing medical magnets on painful areas to reduce soreness and accelerate healing. The actual mechanism by which, and to what extent, magnets affect the body is unclear. Recent experiments involving *Xenopus* embryos indicate that huge magnetic fields of approximately 17 Tesla[1] can change the second and third cleavage planes of development. These planes will orient, vertically or horizontally, to the direction of the applied magnetic field (Denegre, et. al. 1998). The potential effects of magnetic fields on the growth of human fibroblast cells were investigated in this study. Cell cultures were split and the new cultures were exposed to a one-Tesla magnetic field for approximately thirty-six hours during their growth phase. Half of the exposed cultures were counted for proliferation rate and the remainder of the cultures were analyzed for patterns of growth. An F test indicated that there was no significant difference in the growth rates between experimental and control cultures. A Chi square test was used to examine whether cells aligned themselves with the magnetic field during growth. The statistics showed that the data for the control and experimental groups were both significantly different from a random pattern. Since both the control and experimental groups had significant results, it can be concluded that the growth patterns of fibroblasts from the experimental group were no different than those in the control group. The results of this study indicate that magnets do not appear to have an effect on fibroblast growth rates or patterns. This work supports the contention that the reports of positive responses to

[1] One Tesla is equivalent to 10,000 Gauss.

magnetotherapy are due to a placebo effect. It also weakens the argument that electromagnetic fields cause cancer by increasing the growth rates of cells.

Introduction

Magnets, bipolar objects capable of attracting iron, steel, and other metallic items, have varying degrees of strength. The units of measurement for magnets include the Gauss and Tesla. These units are linked with the size of the magnet to determine its pulling power. Various materials are used to make magnets. Ferrite or ceramic magnets contain iron and barium. Neodymium magnets are composed of iron, boron and neodymium, a rare earth metal. The size of the magnet often determines the type of material used for its construction (Welcome to Magnets 4 Health, 2002). Magnets produce magnetic fields that are associated with electric fields. These fields surround all types of electrical equipment and appliances; they are present when the equipment is energized. In the case of an appliance, electric fields are present when it is plugged into the socket, regardless of whether or not it is in use. A magnetic field is absent until the equipment is turned on and the electric current is flowing (Kloepfer, 1993). These electromagnetic fields have been researched for many years to determine if they produce harmful or beneficial effects within the body.

The study of the Earth's magnetosphere has been a difficult task for scientists. Within the past fifty years, the development of spacecraft has made it possible to determine the forces behind the magnetosphere of the Earth and of other planets (Cowley, 1996). The Earth's magnetosphere is formed from two central components: the Earth's magnetic field, generated by currents flowing in the Earth's core, and the solar wind, streaming outward from the Sun at 300-800 km/sec (Cowley, 1996). According to theoretical principles established by Sydney Chapman and Vincenzo Ferraro in the 1930s, magnetic fields are moved by flowing plasmas which are subject to bending and twisting. The same magnetic fields exert forces on the plasmas in order to resist these motions (Cowley, 1996). When the Chapman-Ferraro principles are applied to the Earth's magnetic field, the plasma of the solar wind is associated with the magnetic field produced by all of the planets; the plasma of the Earth is associated with the Earth's magnetic field. When the plasmas encounter each other, they form distinct regions separated by a thin boundary (Cowley, 1996). The Earth's magnetic field does

not continue beyond this boundary. It remains around the planet to form the magnetosphere that provides protection from the solar plasma.

Within the magnetosphere, the magnetic North and South poles produce field lines along which charged particles are driven back and forth. The Van Allen radiation belts are two regions located within these lines. The belts surround the Earth and are composed of many charged particles that originated in the solar wind (The Earth's Magnetic Field, 2002). These particles produce the Northern and Southern Lights at the poles. Another interesting component of the Earth's magnetic field is the poles' ability to reverse direction. Evidence to support this theory includes the study of the magnetic fields of fossil rocks. These rocks show a specific magnetic orientation depending upon the time of formation. A third important characteristic of the Earth's magnetic field is it strength. The magnetic field is essential in protecting the Earth from solar wind and it deflects most harmful radiation. This field is utilized by organisms, such as birds, as an aid in migration. Many behavioral scientists have studied the natural migratory patterns of animals to determine how they use the magnetic field. In 1966, Wolfgang Wiltschko observed the effects of a magnetic field on caged birds during the time of normal migration. He altered the direction of magnetic south and found that the birds gathered in the new direction (Theoretical and Computational Biophysical Group, 2002b). Research on rainbow trout has determined the presence of magnetite-like particles in the brain. The cells located near these particles are connected to a nerve that is triggered during trout exposure to a magnetic field (Travis, 1997). Scientists at the University of Illinois have found a blue-light photoreceptor, identified as cryptochrome, which may explain how animals use the Earth's magnetic field in migration (Theoretical and Computational Biophysical Group, 2002a). This receptor is involved in controlling an animal's day-night rhythm. It is possibly the site of a neurochemical reaction that allows birds to use visual clues from the magnetic field to remain on course (Theoretical and Computational Biophysical Group, 2002a). Experiments indicate that light is essential for the bird to respond. Scientists have found that the light must have a short wavelength such as blue-green. If the light has a long wavelength, the bird cannot orient to the field (Theoretical and Computational Biophysical Group, 2002a). If the Earth's magnetic field disappears, many animals will not be able to navigate in their environments.

The controversy regarding the ill effects of electromagnetic fields (EMF's) began in 1979 when Nancy Wertheimer and Ed Leeper claimed to have discovered a connection between childhood leukemia and power lines. These researchers studied EMF exposures of Colorado children who died of cancer from 1950 to 1973. They reported that children residing in high exposure homes were two to three times more likely than those from low exposure homes to develop cancer, particularly leukemia (Pool, 1990). Scientists discovered several flaws within the study. The primary error was found in the method used to determine exposure to EMF's. Rather than measure the EMF strength, the scientists estimated the value by referring to wire code information that correlated with the types of power lines located near the homes. This information has been found to be an inaccurate indicator of actual EMF strength. Also, the study was not blind, because the researchers knew if cancer patients lived in the homes. David Savitz, at the University of Colorado Medical School, completed a cancer-EMF study in 1988. After he was asked to replicate Wertheimer's work, Savitz found similar results and used statistical analyses to support his conclusions. However, he was questioned due to the small sample size of his study. Other experiments by many researchers were completed, but the results varied from evidence supporting a possible link to the absence of a connection. The risk of cancer was associated primarily with children. Few studies had reported a correlation between adult cancer and exposure to power lines. Adults had an increased risk of cancer if they were electrical workers and spent long periods of time near high frequency EMF's. Many people are skeptical of this research. An article in *Science* suggests that if a link exists between EMF's and cancer, the connection should be supported by higher numbers of childhood leukemia cases due to the increase in electricity use of the past several decades (Pool, 1990). If 30-40% of childhood cancers are caused by EMF's, these types of cancers must have experienced a major increase during the last forty years. Epidemiologists do not agree on how cancer rates have changed over time, but it would be difficult for them to miss a large trend (Pool, 1990). In 1997, Martha Linet, M. D., and a group of researchers attempted to end the cancer-EMF debate. Their study, unlike previous research, included a large number of children. The controls were chosen based upon similarities to the cancer patients. Actual measurements of EMF's were taken in several rooms of the homes as well as in former residences. The technicians

who measured the fields were not informed of the health status of the residents (Linet et. al., 1997). The study showed that the risk of acute lymphoblastic leukemia did not escalate with increasing EMF levels in the children's homes (Campion, 1997). These levels were extremely small when compared to the Earth's static magnetic field of 0.5 Gauss. Some scientists believe that the connection between EMF's and cancer possibly results from factors that have not been considered in the studies. Since high wire code homes are often located on heavily trafficked streets, air pollution may have a role in the leukemia link (Kaiser, 1996). Consequently, many researchers feel that studies on EMF's and cancer should be discontinued. More effort is needed to find definite origins and improved treatments of the disease.

Despite the controversy surrounding EMF's, magnets have been used to treat various medical conditions including arthritis and migraines. Magnetotherapy is the term coined for this alternative approach to medicine. Medical magnets are placed on painful areas to reduce soreness and accelerate healing (Welcome to MagnetTherapy.com, 2002). Numerous companies have taken advantage of this new idea and mass production of magnets designed to aid all body parts has occurred. Jewelry, pillows, and mattresses containing magnets are now manufactured as well as body wraps and shoe insoles. Individual magnets are also produced for use on specific areas of the body. Different sizes and strengths are available for the consumer. One company, magnettherapy.com, sells anti-nausea wrist straps and anti-aging sleep masks in its "Unique and Unusuals" section. Magnets can be obtained for pets such as a magnetic pet bed and body wraps for horses. A new area of magnetic products includes items which magnetize food and water such as the Magnetic Water Muddler sold by Magnets 4 Health.

While these companies have produced magnets for everyday use, medical professionals have developed several types of magnets for use in reducing bone loss and healing fractures. In the 1950s, scientists determined that bones are piezoelectric, indicating that bending or deforming the crystal structure creates local electric currents. Physiologists believe these currents explain why exercise strengthens bones and immobilization weakens them (Raloff, 1999). The OrthoLogic 1000 Bone Growth Stimulator is a portable, noninvasive machine worn by a patient for 30 minutes each day and it provides local magnetic field treatment (Orthologic, 2002). The company has had

a very high success rate with this FDA approved product. The OL1000 decreases a patient's need for surgery to correct a non-healing fracture by combining magnetic fields (dynamic and static) to speed bone growth. Another product, the SpinaLogic Bone Growth Stimulator, also uses these types of magnetic fields to aid a patient's healing process. New studies are experimenting with bone growth stimulators which can reduce osteoporosis. At Creighton University School of Medicine, a model has been produced which requires a person to stand on a platform twice a day for ten-minute sessions. During the sessions, the platform produces electric and magnetic fields. This treatment is anticipated to reduce bone loss (Raloff, 1999). These new machines and products that utilize EMF's as a means to improve health challenge previous evidence indicating that EMF's are damaging forces.

The actual mechanism by which, and to what extent, magnets affect the body is unclear. Recent experiments involving *Xenopus* embryos indicate that magnetic fields of approximately 17 Tesla can change the second and third cleavage planes of development. These planes orient vertically or horizontally to the direction of the magnetic field. The magnets affect the position of the mitotic spindle apparatus. The researchers hypothesize that the magnetic field applies an additional torque to the spindle and astral microtubules (Denegre et. al., 1998). Few scientific experiments have discovered relationships between magnets and the human body. Many positive and negative reports of magnets have been recorded. People who believe magnets are beneficial claim the magnets increase blood circulation by dilating blood vessels and attracting charged particles (Anonymous, 1999a). The blood flow brings more oxygen and nutrients to the area and removes toxic wastes (Welcome to MagnetTherapy.com, 2002). However, Robert Park, a physics professor at the University of Maryland, explains that magnets do not attract blood to a wounded area. Increased blood flow results in reddening of the skin, a symptom that is not evident in the presence of a magnet (Anonymous, 1999b). In association with increased circulation, magnets are also believed to align the water molecules in blood. John Schenck of the General Electric R&D Laboratory in Schenectady, New York states that no magnet exists which could arrange water molecules (Anonymous, 1999b). Other "benefits" of magnets include pH balance and increased hormone production such as melatonin.

Several negative effects have been associated with magnets. Researchers have found that EMF's interfere with drugs such as tamoxifen, which is used to prevent the reoccurrence of breast cancer. Robert P. Liburdy, a biologist at Lawrence Berkeley (Calif.) National Laboratory, claims that increased levels of EMF's inhibit tamoxifen's ability to decrease the growth rate of cancer cells in test tubes. He also states that EMF's affect the ability of melatonin to stop the growth of breast cancer cells (Raloff, 1997). Some scientists believe that EMF's can increase the risk of cancer, because they cause susceptible cells to undergo increased replication, a characteristic of cancer cells. A study at Michigan State University indicates that immature red blood cells exposed to a low frequency EMF do not mature and replicate repeatedly (Sivitz, 2000). This experiment along with other contradictory studies indicates the need for further research which will determine the true effects of magnets on the body.

Due to the controversy surrounding magnets and human health, I chose to research the effects of magnets on human fibroblast growth. I determined the influence of a strong magnet by growing cell cultures and studying the proliferation rate after a specific time period. I examined the pattern of development by staining the cells and describing the general direction of growth. Though my project did not provide answers to every question about magnetic fields, the results answered basic questions about the effects of magnets.

Materials and Methods

930 ml Dulbecco's Modified Eagles Medium with L-Glutamine
100 ml Fetal Bovine Serum
10 ml Antibiotic/Antimycotic
1750 ml Calcium and Magnesium Free Phosphate Buffered Saline (CMF-PBS)
110 ml Trypsin/EDTA
160 culture flasks
9 ml Trypan blue solution
270 ml Wright/Giemsa stain solution
270 ml absolute methanol
270 ml phosphate buffer solution (pH = 6.9)
270 ml distilled water

76 culture tubes for centrifuge
2 Incubators
Inverted microscope
Light microscope
1 Centrifuge
1 Hemocytometer
70% ethanol solution
Sterile pipets: 5 and 10 ml; pipetors
27 1-Tesla magnets
2 vials of human fibroblast cells
Duct tape
Clothespins
Several meters of 60 lb picture wire
Pieces of wood (fir)
Wire cutters
Saw
Sheet metal screws
Drill

Procedures were the same for each set of fibroblast cells. 23 days were required to study the proliferation rates and growth patterns of the cells. A 1-Tesla magnet was placed under each experimental flask. The control group and the experimental group were positioned in separate incubators.

Proliferation Rates and Growth Patterns

On Day 1, the Dulbecco's Modified Eagles Medium (supplemented with L-Glutamine, 10% fetal bovine serum, and 1% antibiotic/antimycotic) was prepared and labeled DMEM++. Two vials of human fibroblast cells were thawed in a 37°C water bath. The vials were dipped in alcohol before being transferred to the work area under the cell culture hood. For this procedure, five flasks and 20 ml of DMEM++ were needed. The contents of the vials were placed in one flask using sterile pipets. 20 ml of the medium were added. The cells were mixed thoroughly with the medium using the

pipets. 5 ml were transferred to each new flask. Two flasks were used as the source of the control group. The cultures were maintained in a water-jacketed CO_2 incubator held at 37°C and 5% CO_2.

On Days 7, 14, and 21, 1:3 splits were performed (refer to Appendix A for splitting procedure). The cultures were fed DMEM++ every 7 days beginning on Day 4 (refer to Appendix A for feeding procedure). The cultures were checked for growth and contamination each day of the experiment.

After the split on Day 21, 27 flasks were randomly selected for the experimental group and the remaining 27 flasks were used for the control group. The magnets were positioned in the incubator that contained the experimental group (refer to Appendix B for magnet placement [Figure 1]). The magnets were placed such that the central axis of the magnetic field was down the longitudinal center of the culture flask. The flasks were returned to the incubators.

After 36 hours of growth, the flasks were removed from the incubators. 26 flasks (13 control, 13 experimental) were stained. These flasks were used to determine the growth patterns of the fibroblasts. The cells in 28 flasks (14 control, 14 experimental) were counted.

Refer to Appendix C for staining procedure.

Refer to Appendix D for counting procedure.

Refer to Appendix E for procedure for determining growth patterns.

Statistical Analysis

An F-Test Two-Sample for Variances was used to evaluate the proliferation rates of viable and nonviable cells for the control and experimental groups. A Chi square test for each group determined the significance of the observed growth patterns.

Results

The cell count for the control group indicated that 189 viable cells and 86 nonviable cells were present. The cell count for the experimental group indicated that 197 viable cells and 120 nonviable cells were present. The percentage of viable to nonviable cells for the control group was 69% to 31%. The percentage of viable to nonviable cells for the experimental group was 62% to 38%. These values can be

found in Table 1. The data in Table 2 indicate the observed growth patterns for the control and experimental groups.

For a statistical analysis of the proliferation rates, the F-Test Two-Sample for Variances (DF = 6) indicated that the critical value was 5.82. The calculated F value for the viable cells was 2.86 (Table 3). The calculated F value for the nonviable cells was 2.94 (Table 4).

For a statistical analysis of the observed growth patterns (refer to Appendix F for pictures of control and experimental cells [Figures 2, 3]), the degrees from Table 2 were combined according to the values in Table 5. For example, the number of cells observed from 30-39° was combined with the number of cells observed from 120-129°. Tables 6 and 7 contain this data. These tables also show the number of cells which cross only the y-axis.

For evaluation of the observed growth patterns of the control and experimental groups, a Chi square analysis (DF = 8) was used. The critical X^2 value was 15.5. Tables 8 and 9 contain the data for the cells located on both axes: x and y. The calculated X^2 value for the control group was 96.3 (Table 8). The calculated X^2 value for the experimental group was 83.1 (Table 9). Tables 10 and 11 contain the data for the cells which cross only the y-axis. The calculated X^2 value for the control group was 61.6 (Table 10). The calculated X^2 value for the experimental group was 72.2 (Table 11).

Table 1. Viable and Nonviable Cell Counts of Control and Experimental Groups

	Control	Experimental
Viable	189	197
Nonviable	86.0	120
Percent viable	69.0%	62.0%
Percent nonviable	31.0%	38.0%

Table 2. Observed Growth Patterns

Degree (°)	Control	Experimental
0-9	1.000	2.000
10-19	8.000	8.000
20-29	35.00	52.00
30-39	72.00	80.00
40-49	84.00	79.00
50-59	56.00	116.0
60-69	56.00	92.00
70-79	65.00	82.00
80-89	54.00	82.00
90-99	72.00	107.0
100-109	58.00	78.00
110-119	57.00	89.00
120-129	73.00	86.00
130-139	68.00	92.00
140-149	52.00	41.00
150-159	35.00	24.00
160-169	7.000	3.000
170-179	4.000	1.000
Total	857.0	1114

Table 3. F-Test Two-Sample for Variances Viable Cells α = 0.05

	Control	Experimental
Mean	27.0	28.1
Variance	40.7	116
Observations	7.00	7.00
Degrees of Freedom	6.00	6.00
Calculated F Value	2.86	
Critical F Value	5.82	
	Critical > Calculated	

Table 4. F-Test Two-Sample for Variances Nonviable Cells α = 0.05

	Control	Experimental
Mean	12.3	17.1
Variance	26.9	79.1
Observations	7.00	7.00
Degrees of Freedom	6.00	6.00
Calculated F Value	2.94	
Critical F Value	5.82	
	Critical > Calculated	

Table 5. Degree (°) Combination		
Combined °		New °
0-9	90-99	0-9
10-19	100-109	10-19
20-29	110-119	20-29
30-39	120-129	30-39
40-49	130-139	40-49
50-59	140-149	50-59
60-69	150-159	60-69
70-79	160-169	70-79
80-89	170-179	80-89

Table 6. Observed Growth Patterns of the Control Group		
Degree (°)	Number of Cells	
	X-, Y- Axes	Y- Axis
0-9	73.0	31.0
10-19	66.0	30.0
20-29	92.0	38.0
30-39	145	69.0
40-49	152	71.0
50-59	108	39.0
60-69	91.0	36.0
70-79	72.0	24.0
80-89	58.0	25.0
Total	857	363

Table 7. Observed Growth Patterns of the Experimental Group		
Degree (°)	Number of Cells	
	X-, Y- Axes	Y- Axis
0-9	109.0	35.00
10-19	86.00	37.00
20-29	141.0	64.00
30-39	166.0	100.0
40-49	171.0	70.00
50-59	157.0	71.00
60-69	116.0	42.00
70-79	85.00	48.00
80-89	83.00	33.00
Total	1114	500.0

Table 8. Chi Square Analysis of Growth Patterns Control Group X-, Y- Axes Degrees of Freedom = 8

Degree (°)	Observed	Expected	$(O - E)^2/E$
0-9	73.0	95.2	5.18
10-19	66.0	95.2	8.96
20-29	92.0	95.2	0.108
30-39	145	95.2	26.1
40-49	152	95.2	33.9
50-59	108	95.2	1.72
60-69	91.0	95.2	0.185
70-79	72.0	95.2	5.65
80-89	58.0	95.2	14.5
Total	857	857	96.3
Calculated X^2	**96.3**		
Critical X^2	**15.5**		
	Calculated > Critical		

Table 9. Chi Square Analysis of Growth Patterns Experimental Group X-, Y- Axes Degrees of Freedom = 8

Degree (°)	Observed	Expected	$(O - E)^2/E$
0-9	109.0	123.8	1.770
10-19	86.00	123.8	11.50
20-29	141.0	123.8	2.390
30-39	166.0	123.8	14.40
40-49	171.0	123.8	18.00
50-59	157.0	123.8	8.900
60-69	116.0	123.8	0.4910
70-79	85.00	123.8	12.20
80-89	83.00	123.8	13.40
Total	1114	1114	83.10
Calculated X^2	**83.10**		
Critical X^2	**15.50**		
	Calculated > Critical		

Table 10. Chi Square Analysis of Growth Patterns Control Group Y- Axis Degrees of Freedom = 8

Degree (°)	Observed	Expected	$(O - E)^2/E$
0-9	31.00	40.33	2.160
10-19	30.00	40.33	2.650
20-29	38.00	40.33	0.1350
30-39	69.00	40.33	20.40
40-49	71.00	40.33	23.30
50-59	39.00	40.33	0.04390
60-69	36.00	40.33	0.4650
70-79	24.00	40.33	6.610
80-89	25.00	40.33	5.830
Total	363.0	363.0	61.60
Calculated X^2	**61.60**		
Critical X^2	**15.50**		
	Calculated > Critical		

Table 11. Chi Square Analysis of Growth Patterns Experimental Group Y- Axis Degrees of Freedom = 8

Degree (°)	Observed	Expected	$(O - E)^2/E$
0-9	35.00	55.56	7.610
10-19	37.00	55.56	6.200
20-29	64.00	55.56	1.280
30-39	100.0	55.56	35.50
40-49	70.00	55.56	3.750
50-59	71.00	55.56	4.290
60-69	42.00	55.56	3.310
70-79	48.00	55.56	1.030
80-89	33.00	55.56	9.160
Total	500.0	500.0	72.20
Calculated X^2	**72.20**		
Critical X^2	**15.50**		
	Calculated > Critical		

Discussion

When the fibroblasts were studied for proliferation rates, more viable and nonviable cells were found in the experimental group than the control group. The cell counts for the experimental and control groups were converted to percentages in order to compare the data (Table 1). The percentage of viable to nonviable cells was approximately the same for each group. An F-Test Two-Sample for Variances determined if the observed differences in proliferation rates were significant. This test indicated that the experimental group did not have a significantly higher or lower growth rate of viable cells compared to the control group (Table 3). Analysis of the nonviable cells with the F test provided similar results (Table 4). The number of nonviable cells in the experimental group was not significantly different than the control group. The magnets did not seem to have an effect on the proliferation rates of fibroblasts. The results support the study completed by Martha Linet et. al. in 1997. The data dispute the possible link between EMF's and cell proliferation as seen in cancer. This link is based upon the belief that EMF's cause cells to replicate repeatedly. The results also indicate that magnetic fields do not have an effect on fibroblast cell growth and further demonstrate that magnets of low power are unlikely to be cancer causing agents. However, studies do indicate that prolonged exposure to high levels of EMF's can increase an adult's cancer risk (Pool, 1990). The findings of this research dispute the beneficial effects of magnetotherapy. The absence of a change in proliferation rates shows that magnets probably do not have healing properties as they cannot increase the number of cells needed to repair a wound nor can they decrease the growth rate of harmful cells, such as tumor cells.

When growth patterns were initially analyzed, Chi square tests for the cells located on both axes were completed. This analysis included all of the angles (0°-179°). The calculated X^2 values of the control and experimental groups were much greater than the critical X^2 values (data not shown). In order to minimize the difference between these results, the degrees of growth were combined according to the values presented in Table 5. For example, cells growing between 0 and 9 degrees on the y-axis were equivalent to cells growing between 90 and 99 degrees on the x-axis. A second set of Chi square tests was completed on the combined data. For both control and experimental groups, the results indicated that the calculated X^2 values were

greater than the critical X^2 values (Tables 8, 9). The null hypothesis was rejected for both groups. Cell growth occurred in specific angles rather than random patterns. The importance of examining the experimental group as well as the control group was shown in this portion of the analysis. If the data for the experimental group were the only information to be statistically tested, the results would illustrate that there was a significant difference in growth patterns. It would be concluded that the magnets had an effect on cell growth. However, the Chi square test also indicated that there was a significant difference in growth patterns for the control group. Since null hypotheses were rejected for both groups, the results provided evidence that magnets did not have an effect on the growth patterns of fibroblasts. The control group showed that the cells typically grew in specific angles in the absence of magnets.

As a final analysis, a Chi square test was applied to the cells which were present along the y-axis. The basis for this test was the placement of the magnets on the flasks. A magnet was aligned with the x-axis of each flask. If the magnets were to influence the growth of the fibroblasts, the cells on the y-axis would be affected more strongly than the cells along the x-axis. This is due to the magnetic field lines that enter and leave the magnets at the poles. These lines form a sphere. However, fibroblasts grow in a monolayer i.e. the cells do not grow on top of each other. Fibroblasts located along the y-axis are subjected to more magnetic field lines than the fibroblasts located along the x-axis, because the latter group is aligned with the magnet. The results of the Chi square test indicated that the calculated X^2 values were greater than the critical X^2 values for the control and experimental groups (Tables 10, 11). The null hypotheses were rejected, illustrating that there was a significant difference in the growth patterns of both groups. Certain angles were observed more than others. This is most likely due to the fibroblasts' tendency to develop in groups. When the cells divide, the new cells often grow in the same direction as the original cells. Clumps of individual fibroblasts growing at the same angle were observed in the flasks. Since the control group experienced the same results as the experimental group, the data demonstrated that the magnets did not cause the observed growth patterns. Studies of *Xenopus* embryos have indicated that high power magnets can cause cells to grow in specific directions (Denegre et. al., 1998). These magnets of 17 Tesla are extremely powerful when

compared to the 1 Tesla magnets of this research. Inducing changes in the growth patterns of fibroblasts may be possible only in the presence of high power magnets.

This study demonstrates that magnets do not have an effect on proliferation rates or growth patterns of fibroblasts. The research weakens the argument that EMF's and magnets increase the risk of cancer by causing vulnerable cells to replicate uncontrollably. No significant difference between proliferation rates of experimental and control cells is observed. Since the magnets are not influencing the cell cycle, they are not predisposing the cell to become cancerous. The study also supports previous research which finds that magnets do not cause biological events such as increased blood flow. This analysis of fibroblasts provides evidence that the reports of positive responses to magnetotherapy are possibly due to a placebo effect. However, it should be considered that these results are subject to change with the use of a higher power magnet as indicated by the work of Denegre et. al.

Acknowledgments

I would like to thank Dr. Glenn Stokes for his advice and assistance throughout the course of this project, Dr. William Birkhead for his helpful comments, and my parents, David and Kathy Chaplin, for their support.

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Appendix A

Use a sterile technique for cell splitting and feeding.

Procedure for Cell Splitting (1:3):

1. Gather the supplies required for cell splitting: DMEM++, CMF-PBS (buffer), Trypsin, three culture flasks, sterile pipets, waste beaker.
2. Use a 70% ethanol solution to sterilize the work area under the cell culture hood.
3. Sterilize the different bottles before placing them in the work area.
4. Using an aseptic technique, open the culture flask which contains the cells. Pour the medium into a waste beaker.
5. Pipet 5ml of CMF-PBS into the flask and swirl it carefully before emptying the buffer into the beaker.
6. Pipet 1ml of Trypsin into the flask. Swirl the flask and place it flat on the counter. Allow approximately ten minutes for the cells to detach from the bottom of the flask. Before completing the next step, the flask should be examined to ensure that the cells are no longer attached to the flask.
7. Pipet 15ml of fresh medium into the flask. Use the pipet to draw up the liquid and spray the cell side of the flask to break the cell monolayer. Spray the side several times.
8. Use the pipet to collect 5ml of the medium and place the liquid in one new flask. Repeat with the remaining 10ml and two other flasks.
9. Return the flasks to the incubator and loosen the caps of the flasks.

Procedure for Culture Feeding:

1. Follow steps 1- 5 as described in the procedure for cell splitting excluding the trypsin and three flasks.
2. After emptying the buffer into the beaker, pipet 5ml of fresh medium into each flask.
3. Return the flasks to the incubator and loosen the caps.

Appendix B

Magnet Placement:

The magnets were attached to the incubator shelves using duct tape, 60 lb picture wire, clothespins, and pieces of wood (fir). 9 magnets were placed on each shelf in 3 rows. The duct tape was used to position the magnets. The wire was looped

through holes in the shelves to stabilize each magnet. Clothespins were disassembled and the wooden portions were placed under the wire that looped over the magnet. Not all magnets needed this treatment. Small pieces of fir were cut to fit the width of the shelves. Each piece was placed against the magnets and notched if necessary. Screws attached the wood to the shelves.

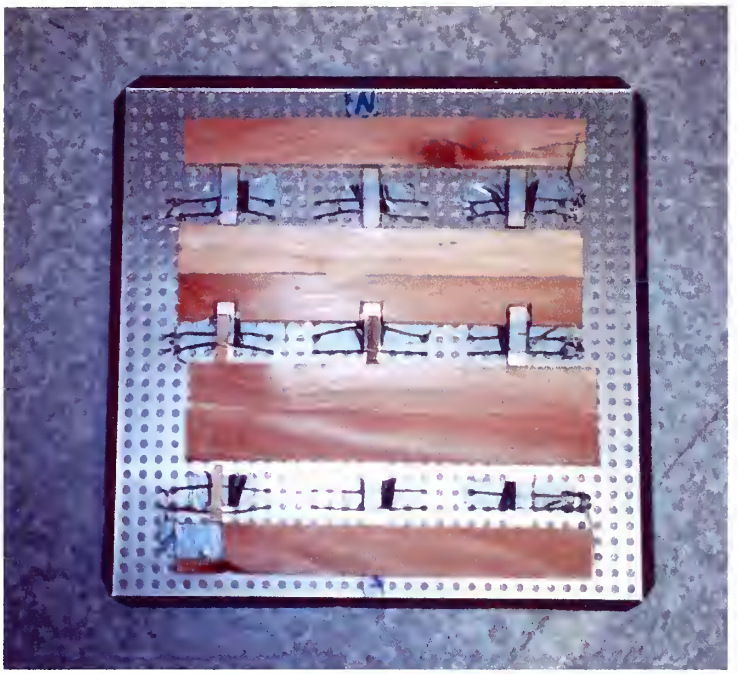


Figure 1. Magnet Placement on Incubator Shelf

Appendix C

Procedure for Cell Staining:

1. Open the flask and pour the medium into the waste beaker.
2. Pipet 5 ml of CMF-PBS into the flask, swirl it, and empty the buffer into the waste beaker.
3. Add 5 ml of absolute methanol to the flask so the layer of cells is covered. Allow the flask to sit for 10 minutes. Cover the flask so the alcohol does not evaporate.
4. Pour the alcohol into the waste beaker and allow the cells to dry.
5. Add 5 ml Wright/Giemsa stain solution to the flask so the entire layer of cells is covered. Leave the solution on the cells for 30 seconds and then empty it into the beaker.

6. Add 5 ml of buffer solution to the flask to cover the cells. Allow the buffer to sit for 3-8 minutes. Pour off the buffer and rinse the cells with 5 ml of distilled water. Allow cells to dry completely.
7. Leave the cells in the flask and view them under a light microscope at 400X and 1000X (oil immersion).

Appendix D

Procedure for Cell Counting:

1. Open the flask and empty the medium into a waste beaker. Pipet 5ml of CMF-PBS into the flask and rinse the cells. Pipet 1ml of Trypsin into the flask and allow it to sit for 10 minutes. Examine the flask under a microscope to ensure the cells have detached from the sides of the flask.
2. Add 10ml of CMF-PBS and use it to spray the cell side of the flask to break the monolayer. Spray several times.
3. Pour the cell suspension into a sterile culture tube. Repeat steps 1 – 3 for all flasks.
4. Centrifuge the tubes at 500 g for 3 minutes.
5. Use a pipet to remove the supernatant from the tube. The liquid can be discarded. Use another pipet to add 5ml of CMF-PBS to the tube to resuspend the pellet of cells. Mix the buffer and cells gently. Combine the contents of two tubes.
6. Remove 0.2ml of the suspension and place it into a clean culture tube. Add 0.3ml of CMF-PBS and mix.
7. Add 0.5ml of trypan blue solution and mix thoroughly using the pipet. The cells are now diluted at a 1:5 ratio (a dilution factor of 5).
8. Place a coverslip on a hemocytometer. Use a pipet to transfer a small amount of the solution to both chambers. Place the pipet at the edge of the coverslip and slowly fill the chambers. Do not overfill or underfill.
9. Choose a starting chamber and count all of the cells (living and dead) located in the center square and four corner squares of the hemocytometer. Keep a separate count of dark (nonviable) and light (viable) cells.
10. Repeat this process on the remaining counting chamber of the hemocytometer.

Mathematical Procedure for Cell Count:

1. Choose one of the five squares in the counting chamber and count all of the cells within the square. For cells located along the edges, count the cells at the top and right edge lines. Repeat for all five squares in both chambers.
2. Each square represents a total volume of 0.1 mm^3 (10^{-4} cm^3). One cubic centimeter equals one millimeter.

Formulas:

Cell concentration

Cells/ml = [(Total number of cells)/(Number of squares)] X dilution factor X 10^4

Total number of cells in the culture

Total cells = [(Number of cells)/(ml)] X original volume of cell suspension

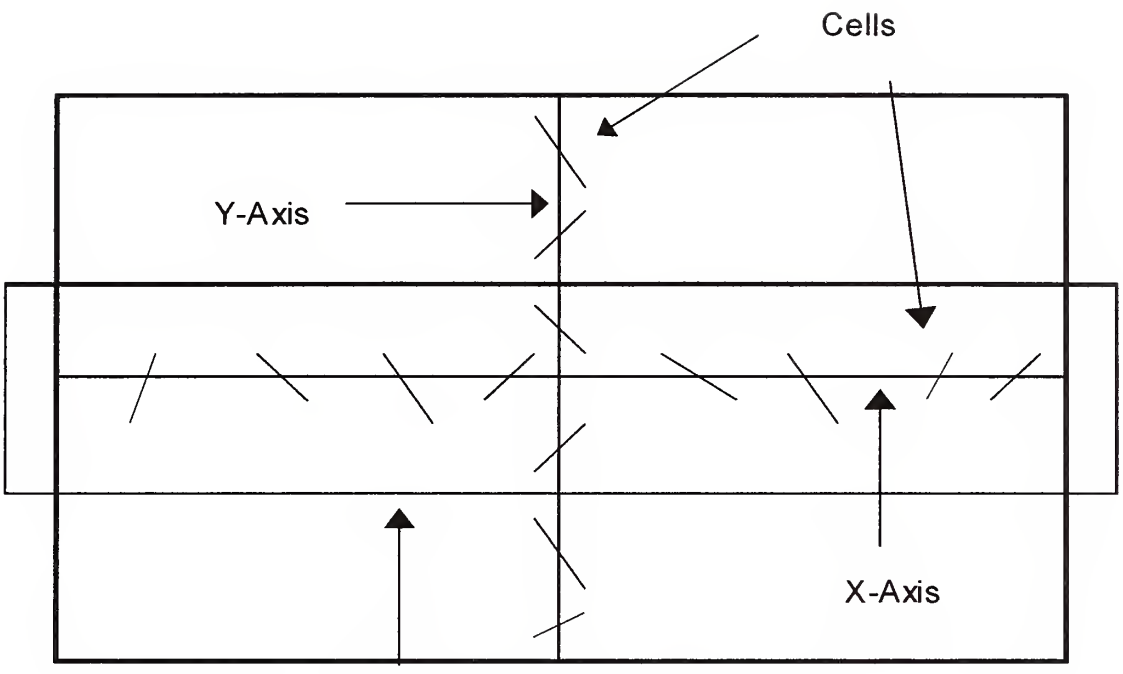
Percentage viability

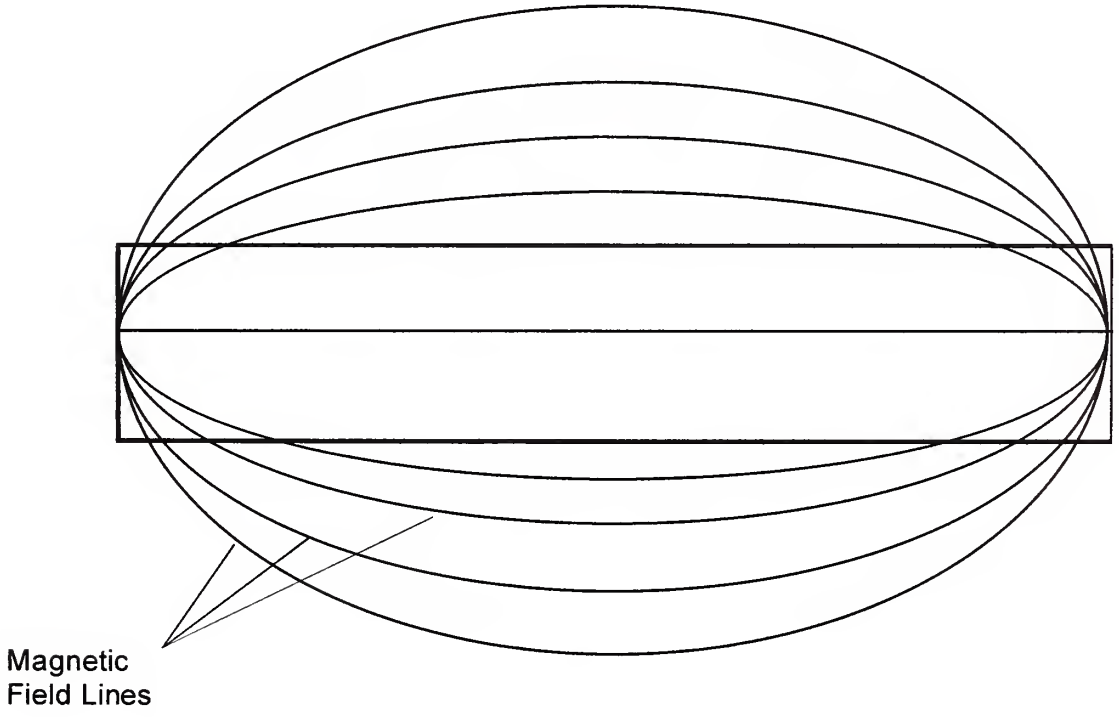
% viability = [(Number of viable cells)/(Total number of cells counted)] X 100

Appendix E

Procedure for Determining Growth Patterns:

Pictures were taken of 26 flasks (13 control, 13 experimental). A grid was placed on each picture to divide it into four quadrants. The cells that were located along each axis were measured with a protractor to determine the angle of growth.


Cells
Y-Axis
X-Axis
Magnet
(1 Tesla)


Magnetic
Field Lines

Appendix F

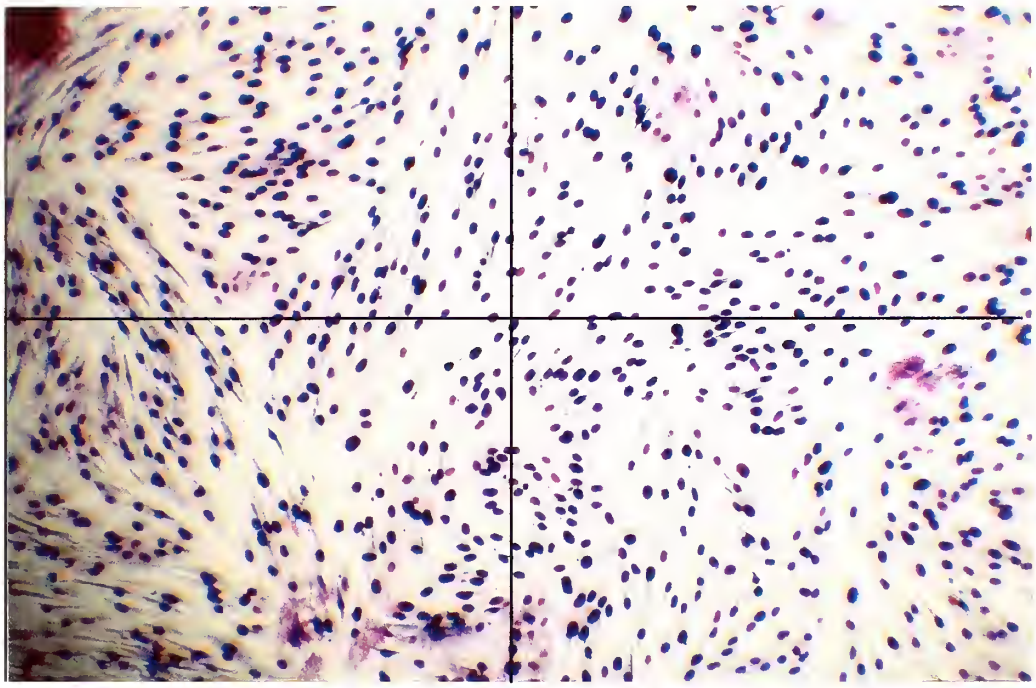


Figure 2. Human Fibroblast Cells Grown for Approximately 36 Hours

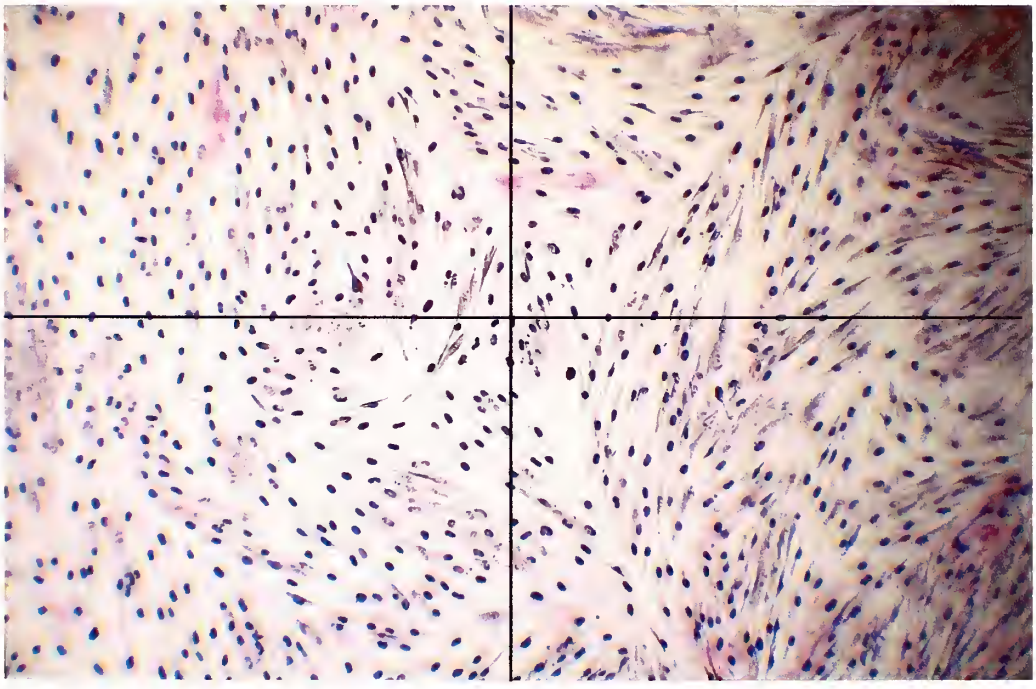


Figure 3. Human Fibroblast Cells Grown in the Presence of a One Tesla Magnet for Approximately 36 Hours