Effect of Manetic Field at Low Frequency on Cells Arrangement

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ABSTRUCT

The effect of the magnetic field at extremely low frequency on cells was investigated *in vitro*. An experimental system of a uniform alternating magnetic field was designed with the Helmholtz coil. Rat's fibroblasts or endothelial cells were exposed to a magnetic field for six days. The results show that there are minor effects of the magnetic field in the intensity level of 0.05 mT on cells at frequency of 50 Hz, and that there are possible effects on the culture medium and on the alignment of the fibroblasts.

Keywords: Bioelectronics, Magnetic field, Cell culture, Low frequency

1. INTRODUCTION

The effects of the magnetic field on a living body were discussed in many previous studies. Some epidemiological researches show possible effects of the magnetic field on human health [1, 2]. Some animal tests were also performed in the previous studies [3]. Another experiments show that the molecules of collagen orient along the static magnetic field [4].

2. METHODS

An experimental equipment was manufactured to generate uniform alternating magnetic field at extremely low frequency.

Helmholtz coil

An alternating magnetic field is generated around a coil with alternating electric current (I). To generate uniform magnetic field, Helmholtz coil was used. The Helmholtz coil consists of two circular coils of which both radius and turns are equal to each other. In the Helmholtz coil, two coils are located in the parallel position with the common axis (X). The distance between two coils (L) is equal to the radius (a) of the coil (Fig. 1). The magnetic field generated between two coils is quasi-uniform (Fig. 2), and the magnetic flux density between them is approximately constant (Fig. 3).

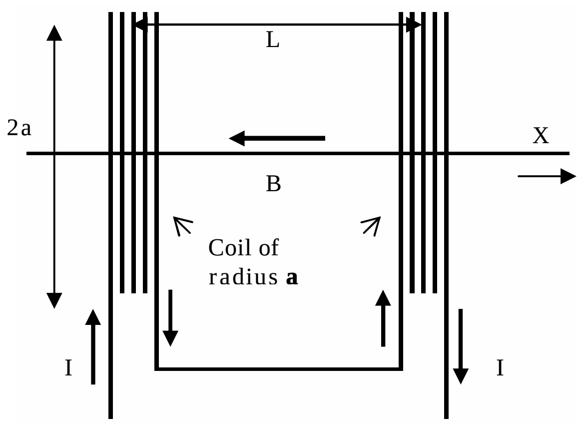


Fig. 1: Principle of coil.

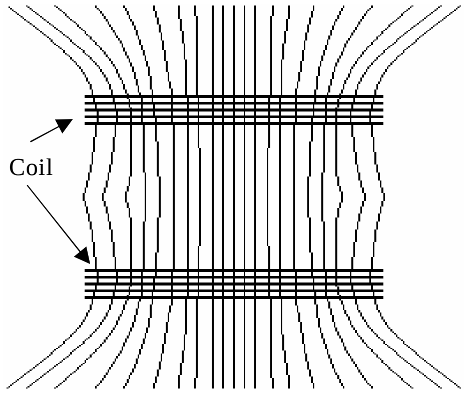


Fig. 2: Magnetic field between two coils.

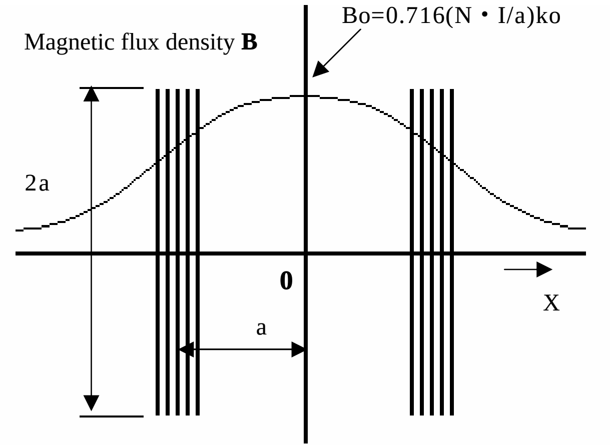


Fig. 3: Magnetic flux of Helmholtz coil.

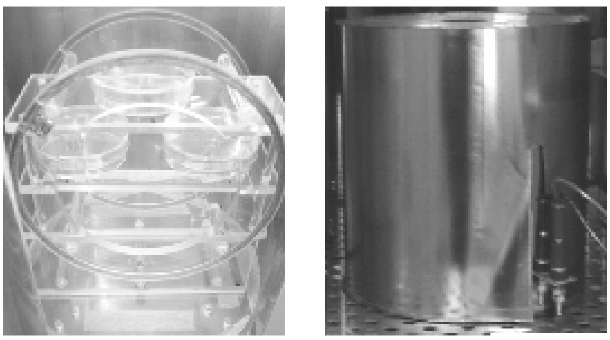


Fig. 4: (a) Helmholtz coil (dishes parallel to the magnetic field), (b) Magnetic shield.

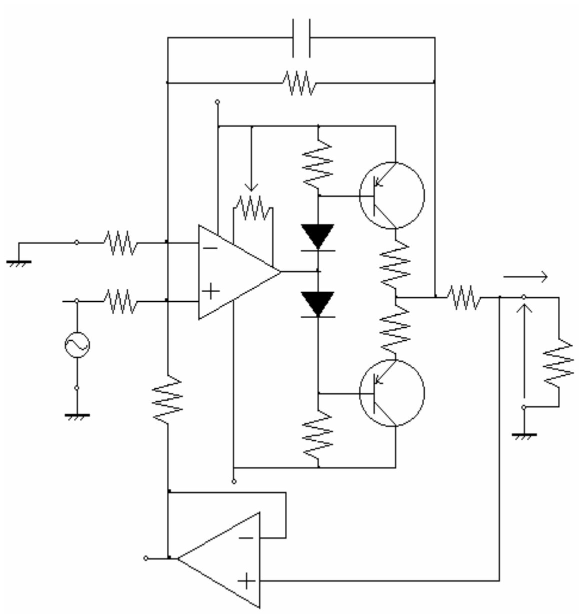


Fig. 5: Circuit diagram of converter from voltage to electric current.

A uniform alternating magnetic field (50 Hz, 0.05 mT) was generated with the Helmholtz coil manufactured with radius of 70 mm (Fig. 4(a)). Four dishes (radius, 30 mm) were placed in the magnetic field so that the culture plane of the dish is either parallel to or perpendicular to the field.

Magnetic shield

The Helmholtz coil was enclosed with the flexible sheet of metal to shield the experimental space from the surrounding magnetic field (Fig. 4 (b)).

Converter from voltage to electric current

Through the converter, the output electric current is controlled with the input voltage (Figs. 5 & 6). The current in the coil is able to be kept independent of load resistance of the coil. The output current varies 0.5 mA with the variation of 1 V in the input voltage. The maximum output voltage of operational amplifier is 10 V, which makes the maximum output current of 5 mA. The offset circuit was added to the converter.

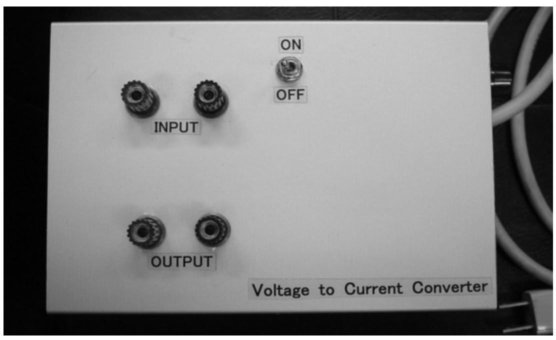


Fig. 6: Converter from voltage to electric current.

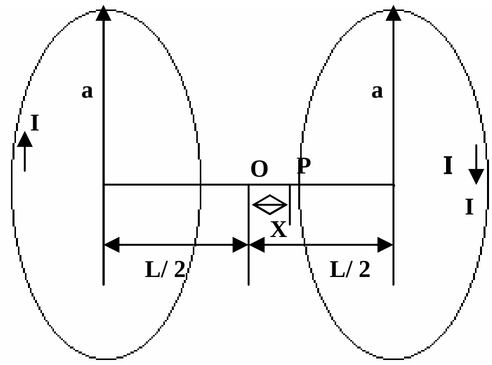


Fig. 7: Two circular coils.

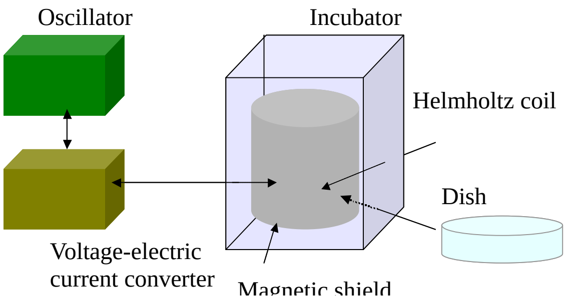


Fig. 8: Experimental system.

Magnetic field between two coils

Two circular coils of radius (a) are located in the parallel position with distance of L at the common axis (Fig. 7). An electric current (I) flows in the clockwise direction at each coil. The magnetic field (H) at the point P, which is away from the center of the axis (O) with distance of X, is calculated by the following equations.

$$H=I a^2/[2\{a^2+(L/2+x)^2\}^{3/2}]+I a^2/[2\{a^2+(L/2-x)^2\}^{3/2}]$$
$$=[I a^2/\{2(a^2+L^2/4)\}^{3/2}]\,[\{1+(Lx+x^2)/(a^2+L^2/4)\}^{-3/2}+\{1-(Lx-x^2)/(a^2+L^2/4)\}^{-3/2}] \quad (1)$$

The magnetic field (H) can be approximated with Eq. (2), when x is small.

$$1 \gg (Lx+x^2)/a^2+(L^2/4),\ (Lx-x^2)/a^2+(L^2/4) \quad (2)$$

$$H=[I a^2/\{2(a^2+L^2/4)^{3/2}\}]\,[2-3\{(a^2-L^2)/(a^2+L^2/4)\}\,x^2] \quad (3)$$

When the radius (a) is equal to the distance (L), the magnetic field (H) becomes

$$H=0.716(I/a) \quad (4)$$

$$B=k_0 H \quad (5)$$

When each coil consists of N turns, the magnetic flux density (B) is calculated with

$$Bo=0.716(N I/a)ko \quad (6)$$

Measurement system

The input voltage generated with the oscillator (Kenwood, AG-D) is converted into the electric current, which flows in the Helmholtz coil. In the experiment, both the magnetic flux density and frequency were controlled with the oscillator. The wave-forms of input voltage and output current are measured with an oscilloscope (Kenwood, CS-5170). The generated magnetic flux density is measured with the gaussmeter (Wandel & Goltermann, EFA-2).

Three sets of the instruments were manufactured and placed simultaneously in an incubator for comparison about three kinds of magnetic conditions: exposed to the parallel magnetic field, exposed to the perpendicular magnetic field, and shielded from the magnetic field.

Cell

Two types of cells were alternatively used in the experiment: rat's fibroblasts L929, or endothelial cells. The endothelial cells were exfoliated from the descending aorta of a rat. The cells were cultured in each magnetic condition for 6 days. The results were evaluated about four points of view: multiplication rate of cells, destruction of cells, the arrangement of cells, and the condition of the medium.

Multiplication rate of cells

The population of cells in the certain area in the dish was counted every twelve hours with a phase contrast microscope. To trace the same area, the plate which has twenty-one holes of 1 mm diameter was attached on the bottom of the dish.

Destruction of cells

LDH (lactate dehydrogenase) is released from a destructed cell. Concentration of LDH was measured with P-L method (Wroblewski method) in the culture medium collected every twenty-four hours from each dish during the test.

Arrangement of cells

The acute angle between the direction of magnetic field and the longitudinal axis of each L929 cell was measured after cultivation for 72 hours. The distribution was compared with that of the direction of longitudinal axes of L929 cells, which were not exposed to the magnetic field.

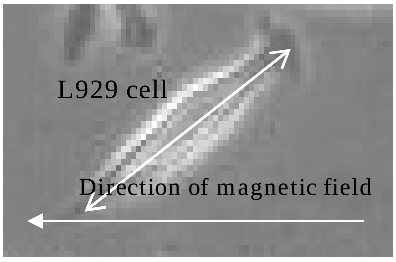


Fig. 9：The arrows show a direction of magnetic field and longitudinal axis of L929 cell.

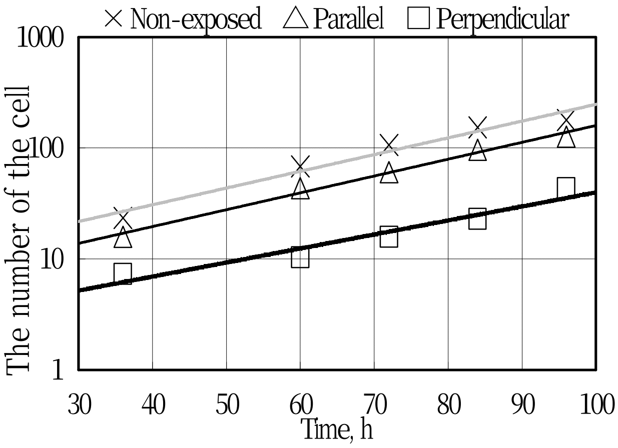
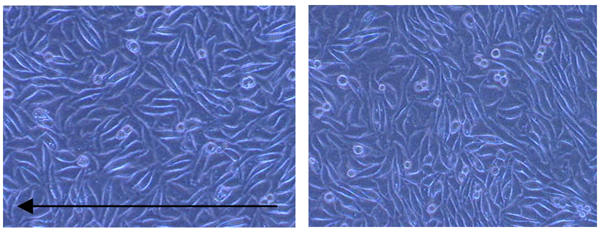


Fig. 10: The rates of multiplication of the three kinds of magnetic conditions (endothelial cells).

Table 1: Concentration of LDH (IU/L at 37 degree C). Time (hour)

Time	parallel				perpendicular			Non-exposure		
	1	2	3	4	1	2	3	1	2	3
24	59	60	60	59	58	60	59	59	57	64
48	72	74	76	73	70	67	72	74	75	73
72	85	86	90	87	85	87	87	91	91	86
96	85	86	90	87	85	87	87	91	91	86
120	19	36	39	42	35	34	43	40	36	36
146	41	32	36	32	37	37	41	38	36	33



(a) (b)

Fig. 11: L929 cells after 72 hours of cultivation: (a) Exposure to the parallel magnetic field, (b) Non-exposure. The direction of the magnetic field is from right to left.

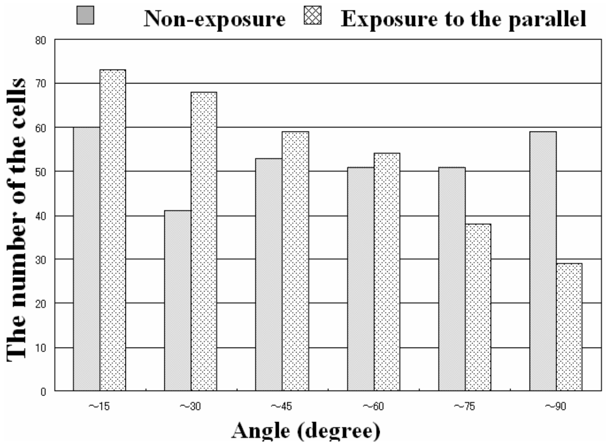


Fig. 12: Distribution of the angles.

Condition of the medium
The color of the medium solution in which cells are cultured changes with its pH. The change shows variation of contents in the medium. The change of the color was measured with intensity of transmitted light through the solution. The intensity was measured every twenty-four hours with the spectrophotometer (Shimadzu, UV-1200) relative to that of distilled water as 100 percent. The deference between intensity of medium exposed to magnetic field and that of medium shielded from the magnetic field was calculated.

3. RESULT

The intensity of surrounding magnetic fields sufficiently decreased with the flexible sheet of metal.

Multiplication rate of cells
Each point in Fig. 10 shows mean value of population in six areas. The population of endothelial cells increases exponentially with time. The slopes of three lines are similar, which shows the similar multiplication rate in three kinds of magnetic conditions: exposed to the parallel magnetic field, exposed to the perpendicular magnetic field, and shielded from the magnetic field.

Destruction of cells
Concentration of LDH does not significantly vary with the three magnetic conditions (Table 1).

Arrangement of cells
Figure 11 shows L929 cells after 72 hours cultivation. The distribution of angle between the direction of magnetic field and the longitudinal axis of each L929 cell is shown with division of every 15 degrees in Fig. 12. The angles of 315 and 321 cells were measured for two magnetic conditions: shielded from the magnetic field and exposed to the parallel magnetic field, respectively. The angles distribute randomly in the cells shielded from magnetic field, where they distribute at smaller division in the cells exposed to the parallel magnetic field.

Table 2: The average angle.

	The number of the cells	Mean angle
Non-exposure	315	45.4
Exposure to paralell	321	37.1

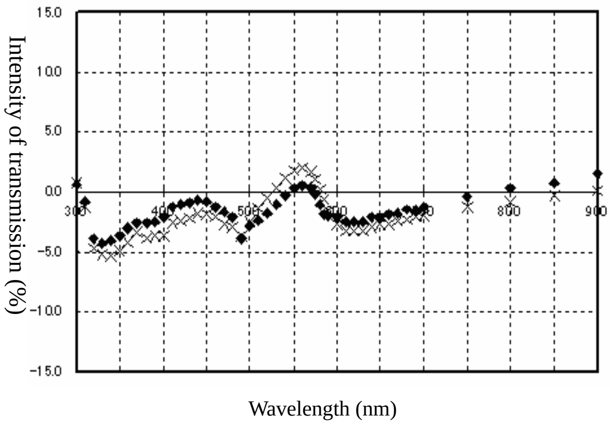


Fig. 13: Difference of transmittance of the medium. L929 cells after 120 hours of cultivation.
×: exposure to perpendicular ◆: exposure to parallel.

The mean angle of cells exposed to the parallel magnetic field is smaller than that of cells shielded from magnetic field (Table 2). The result shows that the cells tend to align with the direction of the magnetic field.

Condition of the medium

Deference of transmitted light intensity between the medium solution exposed to the magnetic field and the medium shielded from the magnetic field is shown as a function of the wavelength between 300 and 900 nm in Fig. 13. Only small deviations are shown around 350 nm, 500 nm and 650 nm.

4. DISCSSION AND CONCLUSION

The Helmholtz coil is effective to generate the parallel magnetic field. The uniform alternating magnetic field was controlled in the present experimental device with the Helmholtz coil and with the flexible sheet of micro-grain metal. The intensity level of 0.05 mT occurs in the atmosphere of daily urban life.

The lactate dehydrogenase is released from cells during their destruction process, and used as an index for destruction of cells. The concentrations of lactate dehydrogenase in the medium replaced every 24 hours were in the same level in the present experiments. The results show constant low destruction of cells regardless of the magnetic field conditions.

The present experiments show only minor effects of the magnetic field of 0.05 mT at extremely low frequency on multiplication of cells. The difference of transmittance intensity indicates the difference of solutes in the medium, which have not been identified.

To study the effect of the alternating magnetic field on cells, three magnetic conditions were designed with cell culture. The study shows that there are minor effects of the alternating magnetic field in the intensity level of 0.05 mT on cells at extremely low frequency of 50 Hz, and that there are possible effects of the alternating magnetic field both on the cell alignment and on the culture medium.

5. ACKNOWLEDGMENT

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6. REFERENCES

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