OPTIMUM DESIGN OF MAGNETIC FIELD ENVIRONMENT FOR AXONAL GROWTH CONTROL IN NERVE CELL REGENERATION PROCESS USING ELECTROMAGNETIC FIELD ANALYSES

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Abstract. *In this study, an optimum magnetic field environment for the nerve axonal extension and control of axonal growth direction in the nerve cell generation process was searched by using electromagnetic finite element analyses.*

Recently, the developments of 3D-scaffold structures employing biodegradable polymers have been an attracting attention for the clinical treatments of damaged nerve tissues. The magnetic stimulation is introduced to accelerate the regeneration speed of nerve axon inside the 3D-scaffold. According to experimental observation of Blackman, C.F. and his research group (1993) [1], it was found that 50 Hz AC magnetic field has promoted the regeneration of axonal extension in the case of pheochromocytoma cells (PC12). They identified the optimum configuration of the coil and the threshold value of driving current for the initiation of PC12 axon growth. However, they did not evaluate analytically the magnetic flux density and the magnetic field in the cell culture liquid for the PC12 axon growth initiation. Therefore, at first we employed the electromagnetic finite element analyses (FEA) to evaluate the magnetic flux density in the case of Blackman's experiment. Simultaneously, we identified the relative magnetic permeability of Dulbecco's Modified Eagle Medium (DMEM) as 1.01 at 50 Hz. Finally, we obtained the value of magnetic flux density inside DMEM as 4.2μT.

Next, we try to design the configuration of Helmholtz coil, which can generate an optimum magnetic field to stimulate most effectively for PC12 axon extension. It is confirmed that the magnetic field gradient affect the extensional speed of PC12 axon, which can be achieved by setup the one peripheral coil and two coils at the center. We found an optimum configuration of Helmholtz coil to generate the magnetic field environment and fabricate an experimental bioreactor for PC12 cell culture. We examined the effectiveness of magnetic stimulation for PC12 nerve axon's extension quantitatively. Further, we try to find the relationship between the magnetic field gradient and the direction of nerve axon's extension.

1 INTRODUCTION

In this study, we designed an optimum structure of the cell culture device- the magnetic field loading system -, for an effective cell activation and regeneration [1-7]. By using magnetic field analyses using finite element (FE) method, we conducted optimum structure design of a culture device applying Helmholtz coil for generation of uniform magnetic field distribution and magnetic field gradient distribution on the installed culture dish. We carried out experiments to verify the magnetic field on the cell culture device, and we cultured pheochromocytoma cells (PC12) and evaluated the effects of uniform and gradient magnetic field stimulation on the extension of nerve axon.

In recent years, for the regeneration and remodelling of damaged peripheral nerve tissues, developments of 3D-scaffold structures employing biodegradable polymers are attracting attention. The damaged peripheral nerve tissues extend their axon along with polymers inside 3D-scaffold. However, it is extremely important for nerve tissue regeneration to accelerate the speed of nerve axonal extension inside 3D scaffold. To solve this problem, we focused on magnetic field stimulation. According to the experiment of Blackman, C.F. and his research group, [1] 50 Hz AC uniform magnetic field has promoted the regeneration of axonal extension in the case of PC 12 cell. Kimura and his group [8] reported that by using partial magnetic field gradient can control the movement of cell population. Furthermore, Saito and his group [9] reported recently that partial electromagnetic field stimulation causes the change of membrane potential. The change of membrane potential strongly connected with the influx of Ca^{2+} ion inside membrane, therefore, we expected that magnetic field stimulation influences the cell activity and regeneration process.

In this study, we analysed the magnetic field loaded under the condition of Blackman's experiments, in which nerve regeneration effects have observed. After that we designed and generated a magnetic field loading culture device which can achieve both uniform and gradient magnetic field. Using this device, we evaluated the effects of the uniform magnetic field stimulation on the promotion of nerve axonal extension and the magnetic field gradient stimulation for the control of nerve axonal growth direction, respectively.

2 ANALYSES AND DESIGN OF CELL CULTURE DEVICE

For the design of magnetic field loading device and magnetic field analyses using finite element analyses, at first, we identified the relative magnetic permeability of Dulbecco's modified Eagle's medium(DMEM) which is used for cell culture. Second, by using magnetic field analyses, we identified the magnetic flux density based on the experiment of Blackman, C.F., which achieved high regeneration speed of PC12 axon's extension. Third, we introduce about the design of magnetic field loading culture device using Helmholtz coil, based on magnetic field analyses. Next, we conducted the verification experiment of produced magnetic field loading culture device. At last, we conducted culture experiment of PC12 cell by using produced magnetic field loading device.

2.1 Identification of relative magnetic permeability of DMEM[10]

For the identification of magnetic flux density based on the experiment of Blackman, C.F. that achieved promotion of nerve axon's regeneration applying uniform magnetic field stimulation, we analyzed the magnetic flux density inside cell culture dish numerically by

using magnetic field analyses. By using magnetic field analyses, we can predict not only magnetic field distribution, but also magnetic flux density inside culture liquid, DMEM. Then, we identified the relative magnetic permeability of DMEM. The relative magnetic permeability μ is an essential magnetic material property for magnetic field analyses. We show the identification experiment of DMEM in Figure 1. We processed trench as 60 mm outer-diameter and 30 mm inner-diameter on chemical-wood made base, and we inserted an enameled wire made Toroidal coil. The trench inserted Toroidal coil is filled with liquid samples, distilled water, saline, and DMEM. The turns of Toroidal coil is 200 to gain a high inductance L, and the diameter of the Toroidal coil is 10 mm and toroidal diameter is 40 mm. The relationship between inductance of Toroidal coil L and permeability of liquids samples μ is given by,

$$L = \frac{\mu a^2 N^2}{2R} \tag{1}$$

where a is toroidal diameter, N is number of turn, R is diameter of toroidal coil. We measured at several frequency magnitude as 5.0×10, 1.0×10^2, 1.0×10^3, 1.0×10^4, 1.0×10^5, 2.0×10^5 Hz. Comparing the results of inductance with DMEM L' and L'' which means without any liquid sample, we can identify the relative magnetic permeability of DMEM applying the equations below.

$$\mu' = \frac{L'}{L''} \tag{2}$$

2.2 Identification of magnetic flux density by using magnetic field analyses [1,11]

In this study, we evaluate magnetic flux density based on the experiment by Blackman C.F. which reported promotion of nerve axon's regeneration using magnetic field stimulation. To evaluate the magnetic flux density inside culture liquid, DMEM, we did magnetic field analyses using ANSYS 14.0. In the magnetic field analyses, we discretize the next two dimensional time-dependent equation and do a numerical analysis.

$$\frac{\partial^2 H_z}{\partial x^2} + \frac{\partial^2 H_z}{\partial y^2} = \sigma \frac{\partial}{\partial t}(\mu_z H_z) \tag{3}$$

where H_z is magnetic field intensity, μ_z is magnetic permeability, and σ is conductivity.

2.3 Design of magnetic field loading device applying Helmholtz coil

To give uniform and gradient magnetic field stimulation to PC12, we selected Helmholtz coil. Helmholtz coil is mainly used for generating uniform magnetic field inside two coils, however, from outside to inside of two coils, we expected that gradient magnetic field would be generated. Therefore, by using Helmholtz coil, we expected we can generate both uniform and gradient magnetic field and confirm the effects of their stimulation to PC12. Magnetic flux density inside and center of two coils is determined by next equation.

$$B_O = \left(\frac{4}{5}\right)^{\frac{3}{2}} \frac{\mu NI}{a} \tag{4}$$

where μ is magnetic permeability of coil's core material, N is number of turns, I is driving current, a is radius of coil. By using this equation, we designed the Helmholtz coil using magnetic field loading culture device.

2.4 Verification experiment of produced magnetic field loading culture device

We conducted a verification experiment of produced magnetic field loading culture device. By using Gauss meter (Toyo technical Ltd), we measured the magnetic flux density at the center of two coils and magnetic field gradient at the edge of coil.

2.5 Magnetic field loading culture experiment

PC-12 cells were obtained from the System Life Science Laboratory at the department of Medical Life System at Doshisha University. The cells were supplemented with 10% horse serum and 5% fetal bovine serum in each collagen coated 35 mm dish in a 5% CO_2 incubator at 37°C with the addition of NGF(50 ng/ml), at 0 days. Culture time is 48 hours, and the cell suspension density is 6.0×10^3 cells/cm^2. We prepared also control culture dish.

By installing semicircular shaped trench on the culture dish installation part, and eliminating half of convex on the bottom 35 mm dish, we arranged culture system so as not to change the direction of 35 mm dish. Further, we marked 5 black dots 3 black lines at the bottom of 35 mm dish. Based on the black dot, we observed the PC-12 cell at ×100 and ×200 magnification. We took microscopic pictures so that the black line marked on the bottom of 35 mm dish, and we measured the length and direction of PC-12 cells. In this case, the black line is parallel to the direction of magnetic field gradient. For the measurement of length and direction of PC-12 cells, we used software; Image J. Measurement was conducted to the axons which satisfy the next criteria.

1. The cells must not connect with the other cells
2. Measurement must be done only one axon pro each cell
3. If some axons or dendrites are extended from one cell, we must select only the longest one
4. If the axons branch off to some direction, we must select only the longest branch.
5. To measure the direction of axon, we must measure at the tip of axon
6. If the axons branch off to some direction, we must select the longest route, and consider the total of axons length as axons length

3 RESULTS

3.1 Results of identification experiment of relative magnetic permeability of DMEM

We show the identification experiment of each liquid samples in Figure 2. At 50 Hz frequency, relative magnetic permeability of distilled water, saline, and DMEM were respectively 0.996, 0.998, and 1.005. Compared with result of distilled water's and its

published value, we determined the accuracy of the identification experiment is by two decimal places. Then, we introduce the relative magnetic permeability of DMEM at 50 Hz as 1.01.

3.2 Results of magnetic field analyses simulating the culture experiment

We show the outline of magnetic field analysis in Figure 3 and analysis modeling in Figure 4. This analysis modeling is based on the experiment by Blackman C.F.. In the model, the diameter of coil is 200 mm, and inside the coils, a 60 mm culture dish is installed. The number of turns, driving current, and electrical resistivity are respectively 1000, 0.5 $mA_{p\text{-}p}$, and 1.68×10^{-8} Ω·m. To these coils, 50 Hz AC current is loaded. Coil cross section is width 25 mm and thickness 10 mm. The relative permeability of each substance is that DMEM is 1.01 and the other parts are 1.00.

We show the result of magnetic field analysis in Figure 5. This analysis result shows scholar solution of magnetic flux density. From this analysis result, we determined the magnetic flux density inside the 60 mm culture dish as 4.22 μT. This analysis based on the experiment which achieved promotion of nerve axon's extension, so we propose this magnetic flux density figure as optimum magnetic flux density for promotion of nerve axon extension.

3.3 Magnetic field loading device applying Helmholtz coil

We show the specification of Helmholtz coil in Table 1. Further, by applying this specification of Helmholtz coil, we conducted magnetic field analyses again to evaluate the magnetic field distribution inside the coils and magnetic field gradient. Figure 6 shows the analysis modeling based on the designed Helmholtz coil. Figure 7 shows the contour solution of magnetic flux density inside designed two coils. From this analysis result, we confirmed the magnetic flux density from outside to inside two coils as 0.1 μT/mm. In this analysis, the magnitude of current was set so that we can generate 4.22 μT; magnetic flux density at the center of two coils. Furthermore, we cannot conduct a verification experiment inside DMEM, because of the specification of Gauss meter, so we did this analysis under the condition without DMEM. Based on this analysis result, we produced a magnetic field loading culture device with acrylic plate with which we can neglect the effects for magnetic field distribution. We show the outline of produced magnetic field loading culture device in Figure 8.

3.4 Results of verification experiment

Based on the designed specification, when we loaded 5.0 $mA_{p\text{-}p}$ driving current to the Helmholtz coil by function generator (hp Ltd), we confirmed 4.0 μT at the center of two coils. We expect that this is because of the resistivity of terminals and function generator. Therefore, we increased the driving current value 5.0 $mA_{p\text{-}p}$ to 5.74 $mA_{p\text{-}p}$. We confirmed also about the generated magnetic field gradient. Because of the specification of Gauss meter, we increased the driving current enough to measure the magnetic field gradient at the edge part of coil from 5.0 $mA_{p\text{-}p}$ to 133 $mA_{p\text{-}p}$. Magnetic flux density increases proximately with the driving current, so we can evaluate magnetic field gradient at the edge coil by increasing driving current. By this experiment, we confirmed the 0.1 μT/mm magnetic field gradients. This magnetic field gradient figure is approximately same as that of the result of magnetic field analysis.

3.5 Evaluation of magnetic field gradient stimulation to direction of PC-12 cells axon's extension

We show the microscopic observation pictures (×200) in Figure 9. From upper row to bottom row, each picture is with magnetic field gradient, with uniform magnetic field, and control, and we show the histogram of direction of PC-12 cells axon's extension from 12 h to 48 h in Figure 10. About histogram, the vertical axis means frequency of axons and the horizontal axis means direction from 0 degree to 90 degree. We show the mean value of direction of axons in Table 2. From the observation result and histogram as shown in Figure 11, we expect that direction of PC-12 cells axon's with magnetic field gradient stimulation tends to decrease compared with control PC-12 cells in each culture times, and the mean value and median are also decreased.

3.6 Evaluation of uniform magnetic field stimulation to promotion of PC-12 cells axon's extension

We show the average length of axons in Table 3. In the Table 3, from upper row to bottom row indicate respectively, with magnetic field stimulation, with uniform magnetic stimulation, and control. From Table 3, the length of axons with uniform magnetic field was promoted from 8.5% to 20.5% compared to control PC-12 cells.

4 CONCLUSIONS

In this study, we aimed to design the magnetic field loading culture device more quantitatively which attracted attention for a one factor of nerve regeneration, by using magnetic field analysis. By modeling the whole culture device, we succeeded to evaluate the magnetic flux density inside culture dish more quantitatively. At the same time, we identified the relative magnetic permeability of DMEM. Furthermore, we identified the uniform magnetic field inside culture dish and magnetic field distribution, and we confirmed magnetic field gradient numerically. Based on the result of magnetic field analysis, we designed and produced a magnetic field loading culture device with which we can achieve both uniform and gradient magnetic field. We evaluated the effects of uniform magnetic field and magnetic field gradient for promotion and control of axon's extension. By the culture experiment result, magnetic field gradient stimulation suggests that can be a one factor of control axon's extension direction, and uniform magnetic field stimulation enhanced the promotion of PC-12 axon's extension as previous study.

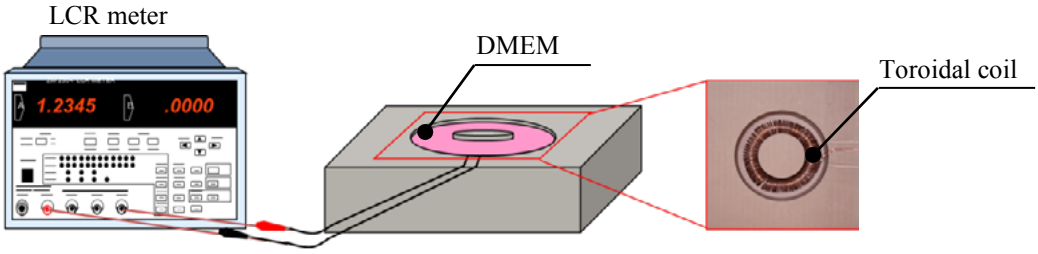


Figure 1: Schematic drawing of relative magnetic permeability identification experiment

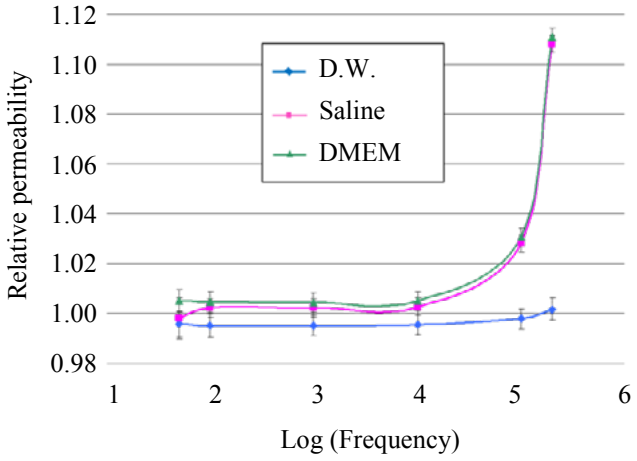


Figure 2: Result of relative magnetic permeabilty identification experiment

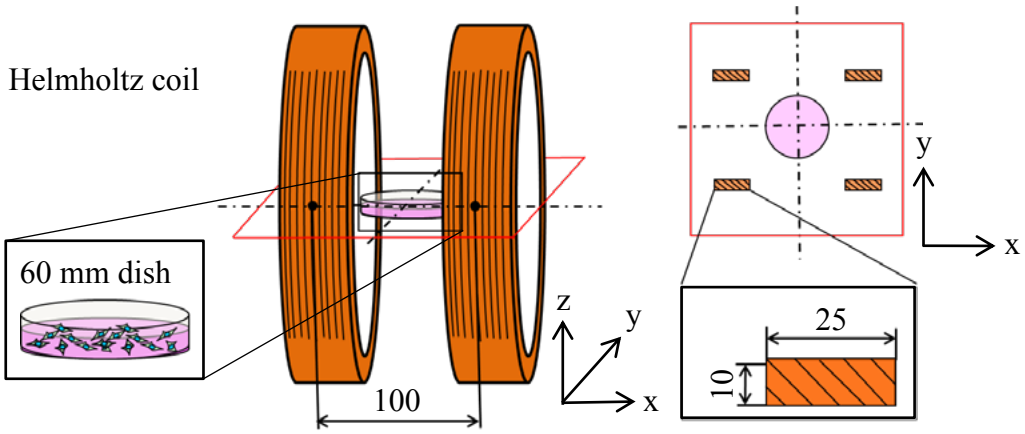


Figure 3: Outiline of 2-dimentional harmonic magnetic field analysis based on Blackman's experiment

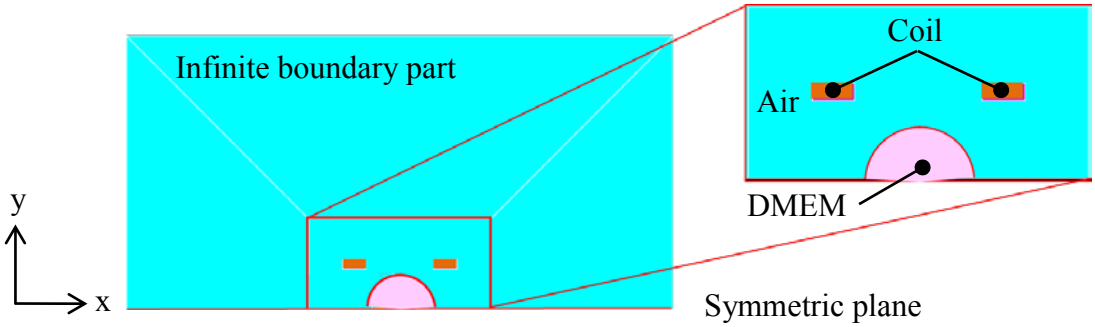


Figure 4: Analysis modeling of 2-dimentional harmonic magnetic field analysis

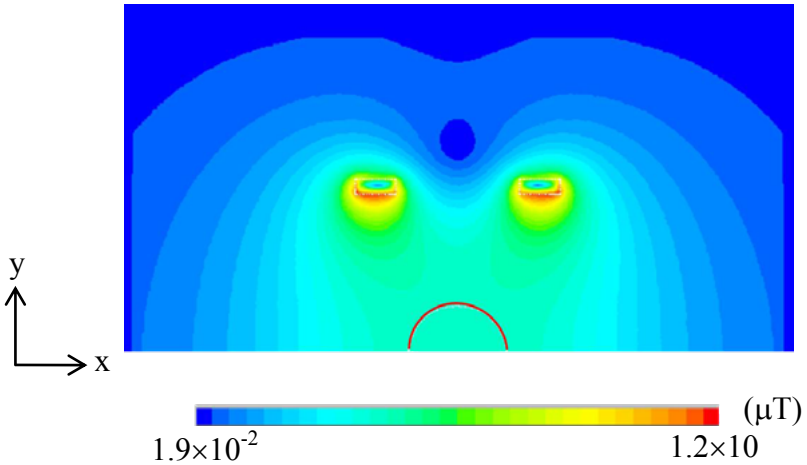


Figure 5: Result of magnetic flux density inside two coils

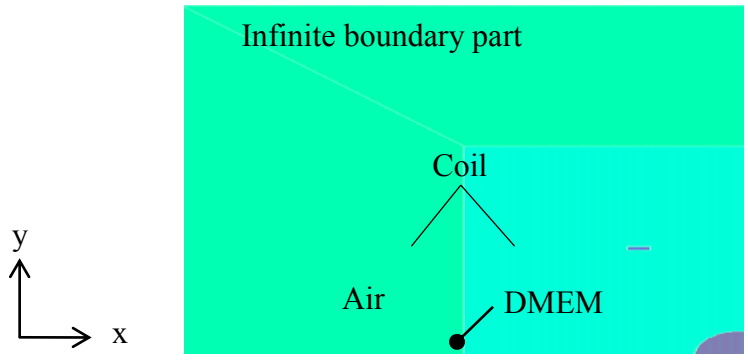


Figure 6: Analysis modeling of 2-dimentional harmonic magnetic field analysis based on design

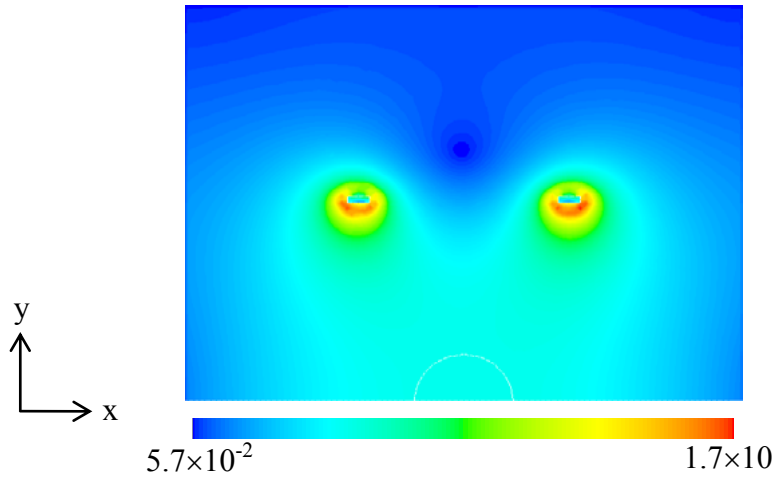


Figure 7: Result of magnetic flux density inside two coils based on design

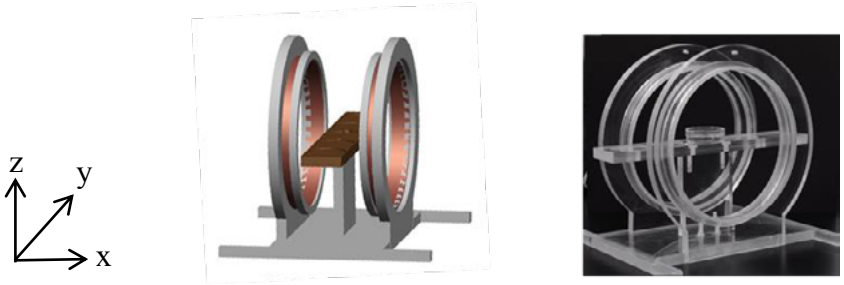


Figure 8: Outline of magnetic filed loading culture device using Helmholtz coil

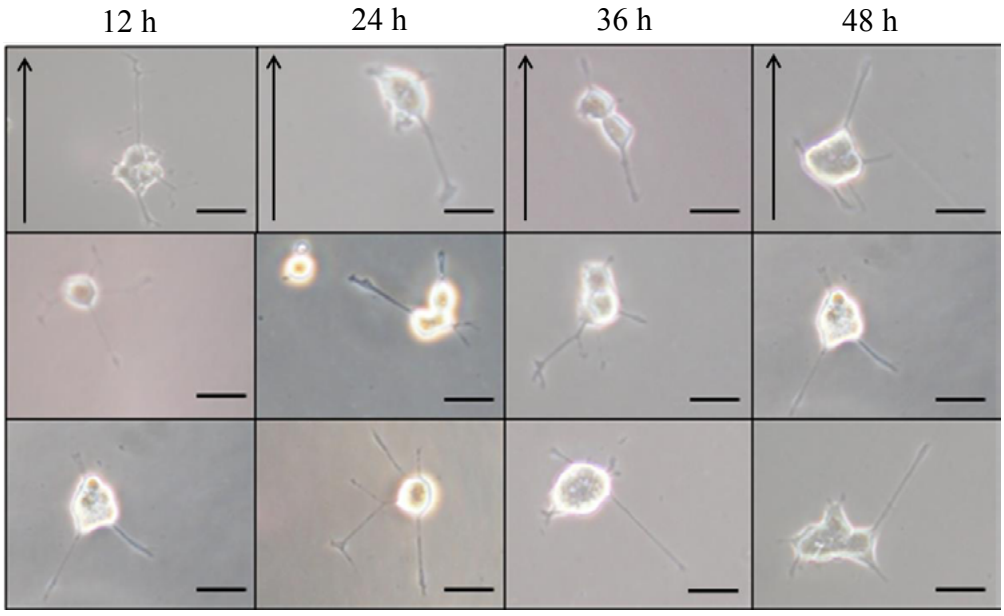


Figure 9: Result of magnetic field loading experiment (Bar = 50 μm)
(Upper row : with magnetic field gradient stimulation, middle : with uniform magnetic field, bottom : control)

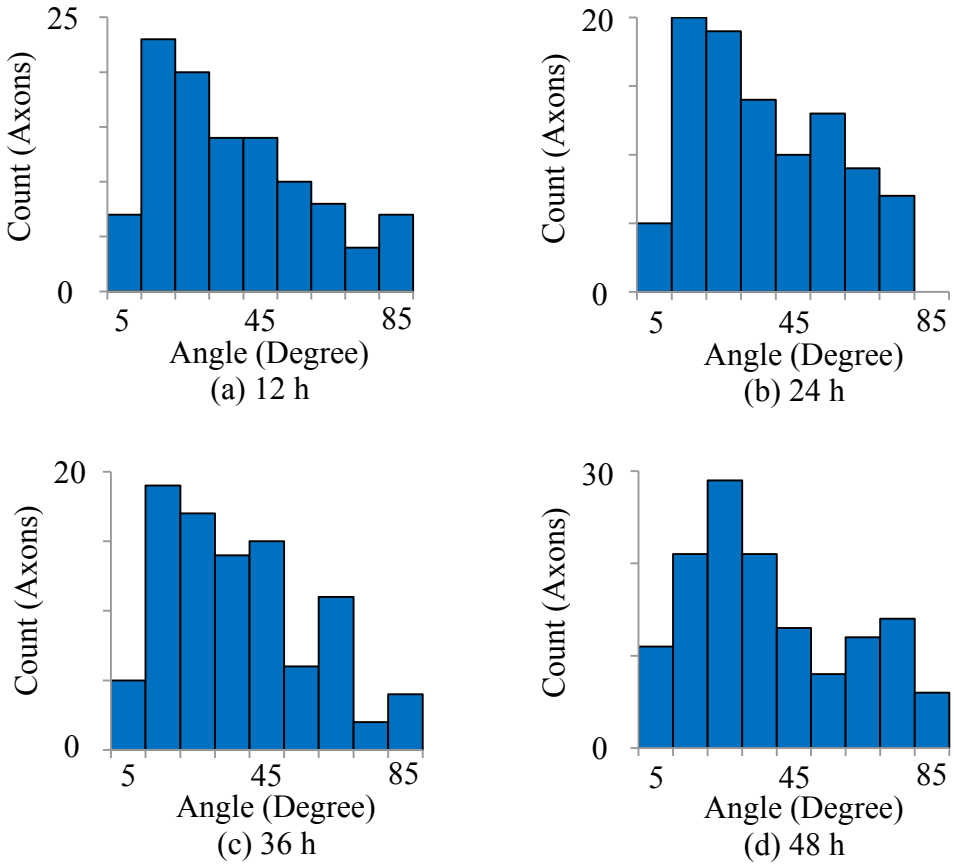
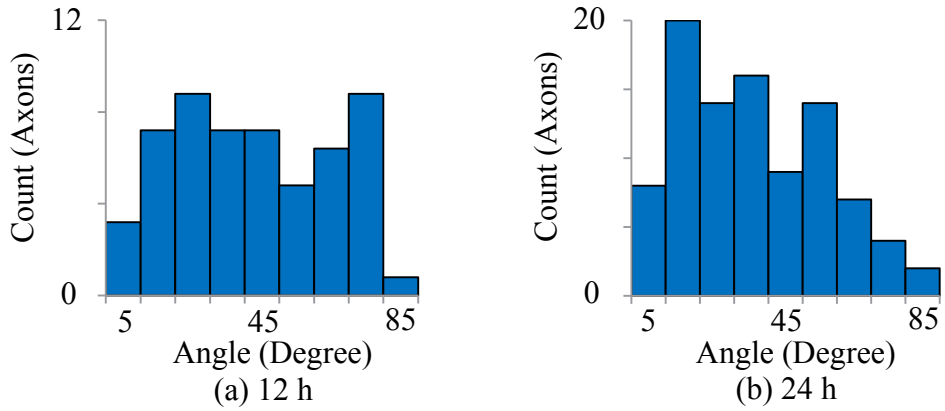


Figure 10: Histogram of axon's extension direction loading magnetic field stimulation



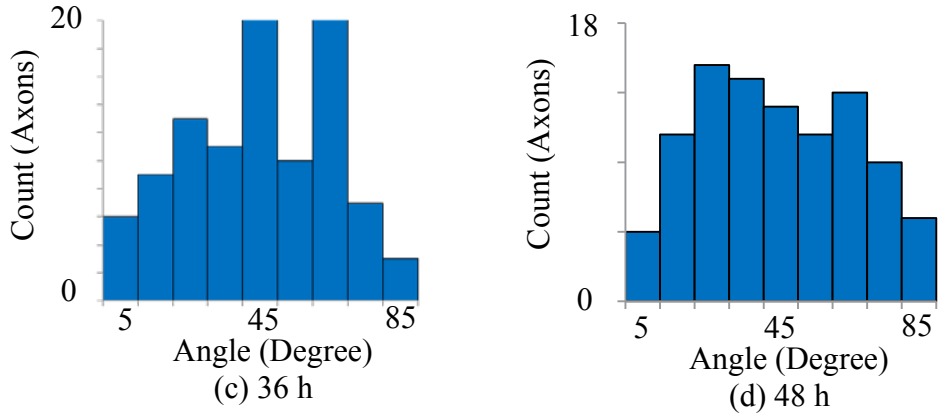


Figure 10: Histogram of axon's extension direction without stimulaiton

Table 1: Specification of desighed Helmholtz coil

Turn *N*	1000
Current *I*	5.0 mA
Diameter *a*	200 mm

Table 2: Average axon's extension direction with and without magnetic field gradient stimulation

	With Magnetic Field Gradient (Degree)	Control (Degree)
12 hours	37.1	42.4
24 hours	36.7	35.5
36 hours	37.0	44.9
48 hours	38.3	44.1

Table 3: Average axon's length with and without uniform magnetic field stimulation

	With Uniform Magnetic Field (Degree)	Control (Degree)
12 hours	30.0	24.9
24 hours	38.1	32.2
36 hours	40.9	37.7
48 hours	41.6	38.0

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