Observations of cellular responses to diamagnetic forces acting on cell components

Masakazu Iwasaka¹, Kentaro Suzuki² and Tadashi Sugawara²

¹ Graduate School of Engineering, Chiba University, Inage-ku, Chiba 263-8522, Japan
² Graduate School of Arts and Sciences, University of Tokyo, Moguro-ku, Tokyo 153-8902, Japan
E-mail: iwasaka@faculty.chiba-u.jp

Received 14 November 2007
Accepted for publication 5 January 2008
Published 20 May 2008
Online at stacks.iop.org/STAM/9/024216

Abstract

The magnetooptical measurements of the properties of living cells have a potentially large impact on cellular engineering and biotechnology because the noninvasive approach to applying magnetic fields on cells enables the detection of the dynamics of intracellular components under natural conditions. In this study, we examine a magnetooptical response in smooth muscle cells exposed to a vertical magnetic field of 5 T. The time course of the linearly polarized light transmittance of cells showed both a gradual decrease and fluctuations during exposure at 5 T. Real-time observations of smooth muscle cells and giant rodlike vesicles revealed that magnetic fields cause morphological changes in the cells and vesicles. In addition, results of the optical transmittance measurement of a fish scale indicate that cellular or tissue components are diamagnetically reoriented by magnetic fields.

Keywords: static magnetic fields, diamagnetism, magnetic orientation, cell, cytoskeleton

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Previous studies with fibrin and collagen proved that peptide bonds, which are involved in the helix structure of proteins and are anisotropic in diamagnetic susceptibility under magnetic fields, contribute to the rotation of macromolecules under static magnetic fields on the order of several Teslas (T) [1–3]. In addition, the cell membrane, which is a lipid bilayer, has a distinct diamagnetic anisotropy in the whole cell. Higashi et al [4] reported that red blood cells and blood platelets orient along the direction of magnetic flux. The effects of strong magnetic fields on adherent cell alignments with [5] and without [6] magnetically aligned collagen fibers have also been reported.

In this study, we focus on the behavior of adherent cells under magnetic fields directed toward the surface of the earth. Colonies of living cells are grown on a substrate, and the effects of magnetic fields on the morphology and optical property of individual cells are observed using two methods.

The magnetooptical properties of smooth muscle cells were investigated by the real-time optical measurement of cells inside the bore of a superconducting magnet. Smooth muscle cells were cultured and observed using a video microscope system during exposure to a vertical magnetic field of 5 T.

Previous studies showed the effects of physical stimulation such as the use of electromagnetic fields and the force of gravity on bone healing. Bone-forming processes can be observed in various types of living tissue. Fish scales are one of the most convenient systems for modeling the human bone system. Osteoblasts and osteoclasts, which are bone-forming cells and bone-dissolving cells, respectively, are easily observed in a thin, transparent fish scale.

In this study, a fish scale is exposed parallel and perpendicular to magnetic fields of up to 5 T, and the
observed changes in magnetooptical properties are found to be associated with bone-reforming processes.

2. Methods

2.1. Cell line experiments

A cell line, namely, smooth muscle cell (A7r5; Dai-Nippon Chemicals Co.), was used for the experiments on the linearly polarized light transmittance of adherent cells on a dish plate (Pyrex glass).

An optical system was used to measure linearly polarized light that passed through the cell culture glass dish to detect changes in cell behavior under magnetic fields. Two polarizing plates enclosed the top and bottom of the dish, and the polarizing directions of the plates were normal to each other. The light output from the analyzer on the top of the dish entered into a photon counting system, which dispersed the light at 500 nm. Smooth muscle cells were exposed to a beam of light (φ10 mm) and stabilized at 37 ± 0.2 °C in the magnet’s bore under ambient magnetic fields until the polarized light transmittance became constant. Then, current was introduced into the superconducting coil and a magnetic field of 5 T was obtained.

2.2. Model of artificial cell

A giant tubular vesicle consisting of 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (POPG), was observed in real time using a microscope during magnetic field sweep-up/down.

2.3. Bone cell experiments

A scale of a goldfish, Carassius auratus, was set in a thin chamber filled with 25 µl of culture medium, and the chamber was set parallel/perpendicular to the direction of a 5 T magnetic field. In the bore of a superconducting magnet, a fiber optic system irradiated light in the direction normal to the surface of the scale. The optical intensity of the light transmitted through the scale was continuously measured at 620 and 690 nm.

2.4. Magnetic field generator and microscope

The above-mentioned optical measurement system was set in a vertical-type superconducting magnet with a maximum field of 5 T. The bore of the superconducting magnet 70 mm in diameter was equipped with a cylindrical water jacket. By closing both edges of the bore with cotton cloth, the temperature inside the bore was maintained at 37 ± 0.2 °C during precise optical measurements.

For real-time images of cells under magnetic field exposures, the images were captured by a CCD-type camera head (Keyence VH-5000). The experiment—real-time observation of smooth muscle cells in the bore of a superconducting magnet—was performed at room temperature (24–25 °C) immediately after the cell was removed from a CO₂ incubator without waiting for temperature stabilization inside the bore that would have required 30 min to reach a stable temperature at 37 °C.

3. Results and discussion

3.1. Polarized light transmittance of cultured cells under magnetic fields

Smooth muscle cells were exposed to a beam of light (φ10 mm), and stabilized at 37 °C in the bore of the magnet at 0 T until the polarized light transmittance became constant. Then current was introduced into the superconducting coil and reached 5 T in 5 min. A rapid increase in the transmittance of polarized light, which originated primarily from magnetooptical (Faraday) effects in the culture medium and cellular layer, was observed during the increase in magnetic field, as shown in figure 1.

During the static magnetic field exposure at 5 T, a gradual decrease in the polarized light transmittance of smooth muscle cell occurred. The light transmittance also showed sudden increases several times. However, there was a clear decrease in the light transmittance when the cells were exposed to...
Figure 2. Optical micrographs of smooth muscle cells with and without 5 T magnetic field.

Although the mechanism responsible for the sudden increase remains unknown, we hypothesized that the clear decrease in the light transmittance is due to the changes in cell morphology or the rearrangement of intracellular components. To check this hypothesis, we used a CCD camera at 25 °C to observe the shape of the cells under magnetic field exposure.

Figure 2 shows optical micrographs of smooth muscle cells with (right) and without (left) magnetic field exposure at 5 T. During the exposure, the cells showed contraction of their pseudopods and then began to form a balloonlike shape. The pseudopods of the cells, which were clearly observed when the magnetic fields were turned off, retracted when the magnetic field was 5 T, as shown in figure 2. In addition, balloon-shaped cells were frequently observed under 5 T. These results indicate that the components of the intracellular cytoskeleton, such as microtubules and microfilaments, change their orientation from being parallel to being perpendicular to the bottom surface because diamagnetic torque forces act on the rearrangement of the cytoskeleton.

A similar phenomenon was observed in a model of an artificial cell—a giant, tubular vesicle that consisted of two types of lipid molecule. The effect of a 5 T magnetic field on the conformation of tubular giant vesicles was investigated. The microscopic observation of the vesicle under a 5 T vertical magnetic field showed a deformation in the shape of the vesicle owing to the diamagnetic anisotropy of the lipid molecules, as shown in figure 3.

Four series of experiments with an individually prepared sample provided evidence that a typical type of giant tubular vesicle shows dissolution during the initial sweep-up. From basis of the video image analysis, we speculated that the vertical magnetic flux bent the tube owing to the diamagnetic anisotropy in lipid molecules standing perpendicular to the long axis of the tube.

Results of the measurements of the transmittance of linearly polarized light in the adhering cells show the diamagnetically induced reorientation and redistribution of cell components. Possible cell component candidates are the cytoskeleton and membrane among others. The transmittance of linearly polarized light at 5 T in smooth muscle cells showed a decrease accompanied by intermittent increases. The overall decrease and intermittent increases in polarized light transmittance were caused by the deformation of the cell membrane, which was caused by the diamagnetic anisotropy of the lipid bilayer. The reason for the intermittent increases was considered to be an increase in the birefringence of the cytoskeleton maintaining the cell’s adhesion, which was the cell’s resistance to forces of diamagnetic torque. The obtained results suggest a new, noninvasive method of detecting cell activity by measuring the mechanical response of molecules inside the cell to forces of diamagnetic torque.

3.2. Goldfish scale with bone reforming model system

A model bone reforming system was measured using a fiber optic system that continuously irradiated light at 620 and 690 nm in the direction normal to the scale surface.

In the absence of exposure to a magnetic field, the light intensity showed a simple increase. In contrast, 5 T magnetic fields parallel and perpendicular to the surface of scale exhibited a decrease and increase, respectively, in the initial exposure period of 15,000 s, as shown in figure 4. It was speculated that the strong magnetic fields oriented the cytoskeletons of the cells in the direction of the magnetic field. The results indicate that the orientation of the magnetic fields to the surface of bone tissue, to which bone cells adhered,
affected the conformation of the bone cells. The magnetic control of conformational changes in bone cells could provide a new method of controlling bone remodeling processes, and be available for applications to the treatment of osteoporosis.

4. Conclusions

We performed experiments with magnetic fields of up to 5 T on (i) the transmittance of polarized light by smooth muscle cells, (ii) the optical transmittance in a goldfish scale, and the real-time observation of both (iii) smooth muscle cells and (iv) vesicles (model of an artificial cell).

1. Under the condition where the axes of both light and magnetic field were perpendicular to the surface of cellular adhesion, the linearly polarized transmittance of adhered cells on a dish plate (Pyrex glass) showed a decrease in light intensity for four thousands of seconds. The light transmittance also showed several sudden increases, which were considered to be a fluctuation of light transmittance caused by cellular activity.

2. In living tissue, namely a goldfish scale, the transmittance of nonpolarized light by the scale under exposure to a magnetic field of 5 T showed an increase and a decrease (time constants = 15,000 s), when the direction of the magnetic fields was perpendicular and parallel, respectively, to the scale surface. These results indicate that cellular or tissue components were magnetically reoriented.

The obtained results indicate that diamagnetically induced changes in the morphology of adherent cells are detected by the real-time optical measurement of cells exposed to magnetic fields of up to 5 T. In particular, the variation in light intensity during a period of one thousand of seconds was a candidate for indicating cellular activity concerning the behavior of the cell components.

Acknowledgments

This work was partly supported by Nakatani Foundation of Electronic Measuring Technology Advancement and by Kanzawa Medical Research Foundation. The author acknowledges the scientific comments of Professors Shoogo Ueno (Kyushu University) and Junji Miyakoshi (Hirosaki University).

References

[1] Torbet J, Freyssinet J M and Hudry-Clergeon G 1981 Nature **289** 91
[2] Torbet J and Ronziere M C 1984 Biochem. J. **219** 1057
[3] Murthy N S 1984 Biopolymers **23** 1261
[4] Higashi T, Yamagishi A, Takeuchi T, Kawaguchi N, Sagawa S, Onishi S and Date M 1993 Blood **82** 1328
[5] Tranquillo R T, Girton T S, Bromberek B A, Triebes T G and Mooradian D L 1996 Biomaterials **17** 349
[6] Umeno A, Kotani H, Iwasaka M and Ueno S 2001 IEEE Trans. Magn. **37** 2909