Effect of Two-times 24 hour Exposures to 60 GHz Millimeter-waves on Neurite Outgrowth in PC12VG Cells in Consideration of Polarization

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Abstract—We investigated the effects of millimeter-wave (MMW) electromagnetic fields on neurite outgrowth in PC12VG cells *in vitro*. We examined the average length of neurites of 160 cells exposed or sham-exposed to 60 GHz MMWs with circular and linear polarizations at average power density of 1 mW/cm^2 for two-times of 24 hour exposure. Four parameters, namely the power density, inhomogeneity of the induced distribution, polarization characteristics, and induced temperature increase due to MMW absorption, were determined and analyzed to characterize exposure conditions extensively. Our results suggested that MMW exposure under these exposure conditions has no significant effect on neurite outgrowth in PC12VG cells.

I. INTRODUCTION

Millimeter waves (MMW) have become one of the attractive communication tools for short-range and high-capacity transmission [1], [2]. Public concern about the potential health effects due to electromagnetic field (EMF) exposures is increasing with the increase of human exposure to electromagnetic waves in daily lives. The effects of weak EMF exposure, especially of weak MMW exposure on human and other mammalian cells are still controversial. Pakhomov reviewed the studies on non-thermal effects of microwaves and MMWs at the cellular levels [3]. Among the reported studies, it was reported that circularly polarized MMW suppressed repair of X rays induced DNA damages on *Escherichia coli* [4]. However the polarization characteristics fed by horn antenna has not been fully described in the culture medium where the adherent cells lie [4], [5].

A PC12 cell line which responds reversibly to nerve growth factor (NGF) has been established from a transplantable rat adrenal pheochromocytoma, and has been widely used as a model of nerve cells *in vitro* [6], [7]. The effects on neurite outgrowth in PC12 cells are considered to reflect some aspect of nerve differentiation and development. In addition *in vitro* experiments are expected to provide means to investigate the effect of long-term exposure to a certain extent if the exposure duration covers the cell cycle. In this viewpoint we investigate the effects on neurite outgrowth in PC12 cells.

We previously investigated the effects of 4 hr exposures to 60 GHz MMWs on the doubling times and the cell viabilities of CHO-K1 cells, which showed no significant effect [8]. We used only linearly polarized MMWs in the previous study [8]. In this study we used both circularly and linearly polarized MMWs to consider the effect depended on the polarizations suggested in [4]. In addition the exposure duration was extended to two-times of 24 hr to examine the effects of long-term exposure. We determined and analyzed four parameters, namely the power density, inhomogeneity of the induced distribution, polarization characteristics, and induced temperature increase to characterize exposure conditions extensively.

II. MATERIALS AND METHODS

A. Descriptions of exposure system

The exposure system used in this study was shown in Fig. 1. Continuous-wave at 60 GHz was generated by a multiplier system (Virginia Diodes, inc., VDI-TX-S143) and transmitted through a conical horn antenna (25.8 mm diameter aperture, Quinstar, QWH-VCCR0Z141) used as a radiator. The generator output power was measured with power meter (Agilent Technology E4419B). The stability of the output power was better than 2.5 % during the operation of 24 hr. The generator output port was a rectangular waveguide with a cross section of 3.75 mm $\times$ 1.88 mm, and was connected to a circular waveguide with a cross section of 3.58 mm diameter operating in the TE fundamental wave modes, through a circular-to-rectangular waveguide transition.

A linear-to-circular fixed polarizer (Quinstar, QWL-60MVF0) was used to convert linear polarization fed by the generator through the waveguides into left-hand circular polarization. Two series of experiments were performed with two exposure apparatuses; one apparatus with the polarizer to excite circularly polarized waves (marked as Circular or C), the other apparatus without the polarizer to excite linearly polarized waves (marked as Linear or L). The polarizer was connected between the circular waveguide and the antenna for apparatus (C).

The culture dishes were placed on a custom-designed stage made of an acrylic resin in an incubator (ESPEC BNR–110), and were centered along the boresights of the horn antennas. The Fraunhofer distance was calculated to be approximately 266 mm, and exposures were conducted under a near-field condition at 90 mm from the antenna to gain the high exposure efficiency per input power. The MMW-exposed culture dish and sham-exposed culture dish (the same conditions except for MMW exposure) were symmetrically set on both sides divided by absorber in the incubator to cultivate both MMW and sham exposed cells simultaneously. The impedance matching layers (polycarbonate, 2 mm thickness) were interposed between the apertures and the dishes at 2 mm from the bottoms of the dishes to suppress the multiple reflection between the dish and the antenna.

B. Cell culture

PC12VG cells derived from a rat pheochromocytoma [9], were cultured in Ham's F-12 medium (Nikken Bio Medical Laboratory, Kyoto, Japan) supplemented with 2.5 % fetal bovine serum (MP Biomedicals, LLC) and 15 % horse serum (Invitrogen, Carlsbad, CA USA) at 37 °C in an atmosphere of 95 % air and 5 % CO_2. The cells at a density of 1.0×10^4 cells/cm^2 were seeded onto 60 mm collagen coated dishes (Becton, Dickinson and Company) with 4.0 ml medium. The culture medium were replaced with culture medium containing 50 ng/ml NGF (2.5S, Invitrogen, CA USA) at 24 hr after cell seeding and the unattached cells removed. The exposure protocols were conducted as follows; the cells were exposed for 24 hr to MMW after the medium replacement, and were then cultured for 24 hr without exposure, and were lastly exposed again for 24 hr to MMW after second medium replacement.

C. Analysis of neurite outgrowth

For quantitation of neurite outgrowth, two photographs were captured with a phase-contrast microscope (Olympus CKX41) in the each of 8 observed regions after a series of the exposure protocols. The internal electromagnetic fields in the medium has a distribution of concentric circle-shaped around the center of the medium at 90 mm from the antenna [8]. The observed regions were selected at even intervals to be circular ring enclosed from 6 mm to 8 mm along the radius from the center of the medium to make the exposure distribution uniform.

We measured the length of neurites of 10 cells randomly selected for each photograph, and decided the rules as follows; the longest neurite of a cell with multi neurites was measured, and the cell with shorter neurites than its diameter was excluded from the analysis. The averaged value of the measured length was finally calculated for 160 cells.

The validity of the results was guaranteed by a blinded experimental procedure. The differences between the mean values derived from two groups were examined by statistical analysis using a two-sided paired Student's t test. The differences between group means were considered significant at p-value < 0.05.

D. Dosimetry

The electromagnetic calculations were carried out using the conformal Finite-Difference Time-Domain (FDTD) solver SEMCAD X v14.8 provided by Schmid & Partners Engineering AG to obtain the electromagnetic field strengths in the culture medium. The model of the apparatus for FDTD calculation is shown in Fig. 2. A source for apparatus (L) generates a fundamental TE_{11} mode at 60 GHz toward the positive direction of the z-axis. The sources for apparatus (C) generate a pair of TE_{11} modes which have orthogonal components to each other at 60 GHz with phase difference $\pi/2$. The calculation region was bounded by perfectly matched layer (PML) absorbing boundaries with 8–10 layers.

The electrical constants of each calculation model are shown in Table I [8]. The surface of conical horn antenna was treated as perfect electric conductor (PEC). All calculations were performed using the FDTD mesh with grid sizes $< 0.07\ \lambda$ (λ is the wave length in each calculation region).

TABLE I
ELECTRIC CONSTANTS ASSUMED IN THE FDTD CALCULATIONS (AT 60 GHZ, ε_r: RELATIVE PERMITTIVITY, σ: ELECTRIC CONDUCTIVITY) [8].

Substructure	ε_r	σ [S/m]
Dish	2.58	0.05
Medium	12.1	69.4
Matching layer	2.75	0.00

The ellipticity was used to quantify polarization, and defined as eq. (1).

$$ellipticity = \frac{|\boldsymbol{E}_{min}|}{|\boldsymbol{E}_{max}|},\qquad(1)$$

where, $\boldsymbol{E}_{max}$ and $\boldsymbol{E}_{min}$ are the maximum value and minimum value of the electric field vector in one cycle.

The power density was assumed to be 1 mW/cm^2, which corresponds to exposure limit for human exposure for general public in the guideline recommended by ICNIRP [10]. The spatially averaged power density was calculated to be 1 mW/cm^2 in the observed regions at the antenna input power of 66 mW. Figure 3 shows the histograms of electric field for apparatus (C) and (L). Table II summarizes statistical values of the calculated electric field and homogeneity, normalized to the input power of 66 mW. In both of cases, the variations of electric field in the bottom layer of the medium were relatively uniform with less than 30 % which meets the suggested homogeneity criteria for *in vitro* experiments [11]. It should be noted that the variation of electric field in the observed regions was more uniform than that in the whole bottom layer of the medium.

Figure 4 and 5 show the histograms and the distributions of ellipticity in the bottom layer of the medium, respectively. Table III summarizes statistical values and homogeneity of the calculated ellipticity. The polarized waves for apparatuses of (C) and (L) have closer to circular and linear polarization in the observed regions than those in the whole bottom layer of the medium, respectively.
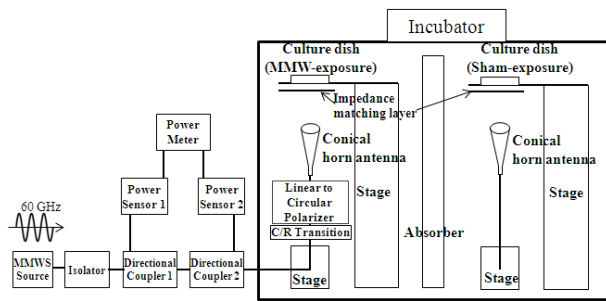
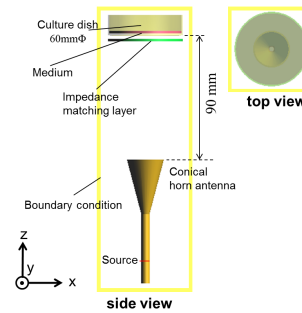Fig. 1. Block diagram of the exposure system.

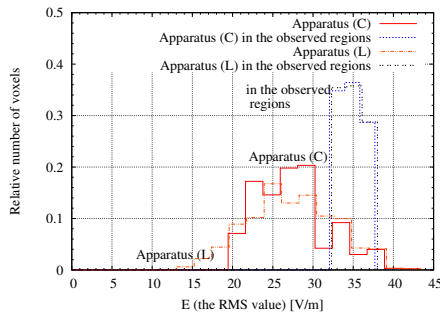Fig. 2. Illustration of the calculation model.

Fig. 3. Histograms of electric field strength in the bottom layer of the medium.
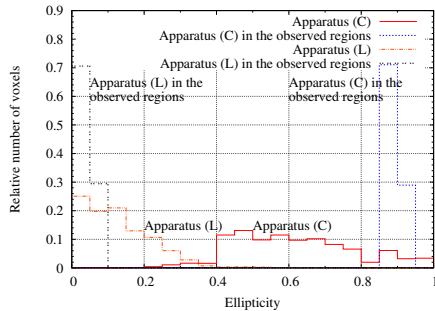

Fig. 4. Histograms of ellipticity in the bottom layer of the medium.

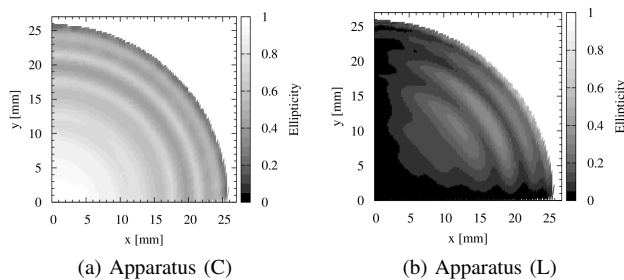(a) Apparatus (C)   (b) Apparatus (L)

Fig. 5. The distributions of ellipticity in the bottom layer of the medium.

E. Temperature calculation and measurement

The temperature increase due to absorbed MMW power was identified numerically. The validations of the calculation results were identified experimentally.

The calculations were performed using the Pennes bioheat transfer equation [12]. The two terms of the power by the

TABLE II
STATISTICAL VALUES OF THE CALCULATED ELECTRIC FIELD (THE RMS VALUE) IN THE BOTTOM LAYER OF THE MEDIUM, NORMALIZED TO 66 MILLIWATTS INPUT POWER. THE RSD STANDS FOR THE RELATIVE STANDARD DEVIATIONS, THE AVE. ARE THE SPATIALLY AVERAGED VALUES, THE MAX. ARE THE MAXIMUM VALUES.

	RSD [%]	Ave. [V/m]	Max. [V/m]
Apparatus (C) (whole bottom)	16.1	27.4	43.2
Apparatus (C) (observed regions)	0.8	34.8	37.7
Apparatus (L) (whole bottom)	19.7	27.4	43.5
Apparatus (L) (observed regions)	0.8	35.1	38.0

TABLE III
ELLIPTICITY IN THE BOTTOM LAYER OF THE MEDIUM. AVE. ARE THE SPATIALLY AVERAGED VALUES.

	Standard deviation	Ave.
Apparatus (C) (whole bottom)	0.17	0.62
Apparatus (C) (observed regions)	0.02	0.89
Apparatus (L) (whole bottom)	0.09	0.13
Apparatus (L) (observed regions)	0.02	0.04

metabolism and blood perfusion were neglected. The absorbed MMW power was the only heating source. We used the convective boundary condition based on the heat flux continuity across the interface boundary to the air-filled external region. The material parameters are summarized in Table IV [13].

TABLE IV
MATERIAL PARAMETERS ASSUMED IN THE THERMAL CALCULATIONS (C: SPECIFIC HEAT CAPACITY, k: THERMAL CONDUCTIVITY, ρ: MASS DENSITY, h: HEAT TRANSFER COEFFICIENT ON THE BOUNDARY) [13].

Substructure	ρ [kg/cm^3]	C [kJ/kg·K]	k [W/m·K]	h [W/m^2·K]
Dish	1100	1.2	0.12	41
Medium	1000	4.2	0.6	7.1

The measurements were performed using a multi-channel optical fiber thermometer (Anritsu Meter Co., Ltd. FL-2000), and remotely controlled via RS232C interface. The meter was calibrated at 37 °C using distilled water with a calibrated thermometer.

The initial ambient temperature in the incubator was 37 °C. The calculated temperature increase for apparatuses of (C) and (L) were 1.1 °C at the center of medium, where the maximum value was estimated to occur, at input power of 66 mW. The results were in good agreement with measured temperature increase. The average values of the calculated

temperature increase in the observed regions were 1.0 °C (standard deviation 0.02 °C) for apparatus (C) and 0.9 °C (standard deviation 0.03 °C) for apparatus (L).

F. Biological experimental setups

Five culture dishes were prepared for each condition, namely MMW-exposure, Sham-exposure, Control (37 °C), Control (38 °C), and w/o NGF. The Control (37 °C) and w/o NGF groups were cultured except for MMW exposures with and without NGF in the same incubator during exposure protocols, respectively. The dish of Control (38 °C) group was moved and cultured except for MMW exposure at 38 °C in other incubator to examine thermal responses due to MMW absorption. Five independent series of experiments were conducted for apparatuses of (C) and (L). The statistical analysis was performed for two exposure groups, two control groups, and between MMW-exposure and Control (38 °C) groups.

III. RESULTS AND DISCUSSIONS

Figure 6 and 7 show the neurite outgrowth in PC12VG cells for apparatuses of (C) and (L) for a forward power of 66 mW with the reflected power of less than 1 mW. All data are expressed by the averaged value and standard error of mean (SEM). The neurite outgrowth of Control (38 °C) group was significantly increased compared to Control (37 °C) group for apparatus (C). The differences between MMW-exposed and sham-exposed groups for apparatuses of (C) and (L) were not statistically significant except for 1 significant difference which may be explained by the temperature increase in the medium. The non-thermal effects on neurite outgrowth in PC12VG cells were not found regardless of polarizations.

However the neurite outgrowth of MMW-exposure group has tendency to be smaller than that of Control (38 °C) group. This tendency may be explained by the presence of a direct effect of MMW exposures on the neurite outgrowth. Alternatively, the tendency may be explained by an indirect effect of MMW exposures due to the different temperature increase in space and time from the temperature increase by ambient temperature in the Control (38 °C) group.
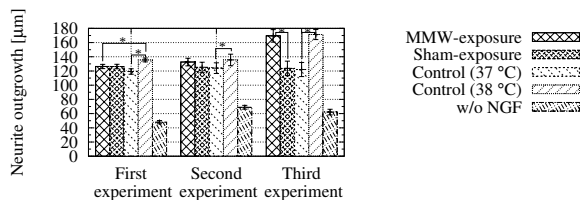

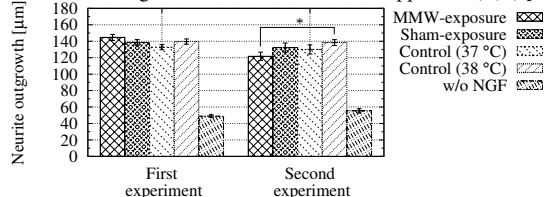Fig. 6. The neurite outgrowth in PC12VG cells for apparatus (C) (∗$p \leq 0.05$).

Fig. 7. The neurite outgrowth in PC12VG cells for apparatus (L) (∗$p \leq 0.05$).

IV. CONCLUSION

We investigated possible non-thermal effects on neurite outgrowth due to exposure to 60 GHz MMWs in consideration of different polarizations. Our results suggested that MMW exposure under the exposure conditions has no significant effect on neurite outgrowth in PC12VG cells.

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