Effects of 60-Hz Time-Varying Electric Fields on DNA Damage and Cell Viability Support Negligible Genotoxicity of the Electric Fields

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Abstract

The effect of a 60 Hz time-varying electric field was studied using a facing-electrode device (FED) and a coplanar-electrode device (CED) for further investigation of the genotoxicity of 60 Hz time-varying magnetic field (MF) from preceding research. Neither a single 30-minute exposure to the CED or to the FED had any obvious biological effects such as DNA double strand break (DSB) and apoptosis in cancerous SCC25, and HeLa cells, normal primary fibroblast IMR90 cells, while exposures of 60 Hz time-varying MF led to DNA damage with induced electric fields much smaller than those used in this experiment. Nor did repetitive exposures of three days or a continuous exposure of up to 144 hours with the CED induce any DNA damage or apoptosis in either HeLa or IMR90 cells. These results imply that the solitary electric field produced by time-varying MF is not a major cause of DSBs or apoptosis in cancer or normal cells.

Key Words: Apoptosis, DNA Double-Strand Break (DSB), HeLa Cell, IMR90 Cell, Time-Varying Electric Field (EF).

I. INTRODUCTION

Extremely low frequency electromagnetic fields (ELF-EMFs) are generated everywhere from activated electric devices and electric wires. ELF-EMFs are composed of electric and magnetic fields whose frequencies range from 1 to 300 Hz. The major frequencies of ELF-EMF, usually used in electric wire transfer and electric devices, are usually 50 or 60 Hz. Humans are continuously exposed to various frequencies of EMF-EMFs in daily life. The biological effects of EMF have already been closely studied, and the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has designated EMF in the MHz-GHz range as potentially carcinogenic [1]. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) has also produced guidelines for all kinds of non-ionizing radiation [2]. However, the physiological effect of low-frequency EMFs has been controversial.

As bioelectrical responses are very slow, the biological effect is considered negligible, except for heating. Therefore, it has generally been believed that alternating current (AC) fields of 100 kHz or above have no meaningful biological effects [3]. However, it was reported by Kirson et al. [4, 5] that low-intensity AC electric fields at a frequency between 100 kHz

and 1 MHz hinder or destroy cell division and decrease the growth of brain tumors at electric field intensities of just 1–2 V/cm. They suggested the mechanism of this observation with the simulation of electric field distribution in a cell during cell division [5].

Previously, we designed a 60-Hz time-varying magnetic field (MF) device and observed that a single exposure to an EMF at 6 mT for 30 minutes induces DNA double-strand breaks (DSBs) and activates DNA damage checkpoints in both the normal fibroblast IMR90 and the cancerous HeLa cell [6, 7]. In addition, repetitive exposures of HeLa and IMR90 cells for 30 minutes per day for 3 days led to apoptosis [6]. However, we could not conclude whether these genotoxic effects of EMF were mainly due to the time-varying MF itself or due to the electric field (EF) induced by the time-varying MF.

In this study, to understand what is responsible for the genotoxic effects of time-varying MFs, we designed two devices to generate EFs parallel or perpendicular to the surface of a culture dish, and we examined the effects of an EF on primary and cancer cells. One device was powered by a 60-Hz sinusoidal and the other by a 60-Hz bipolar pulsed voltage.

II. MATERIALS AND METHODS

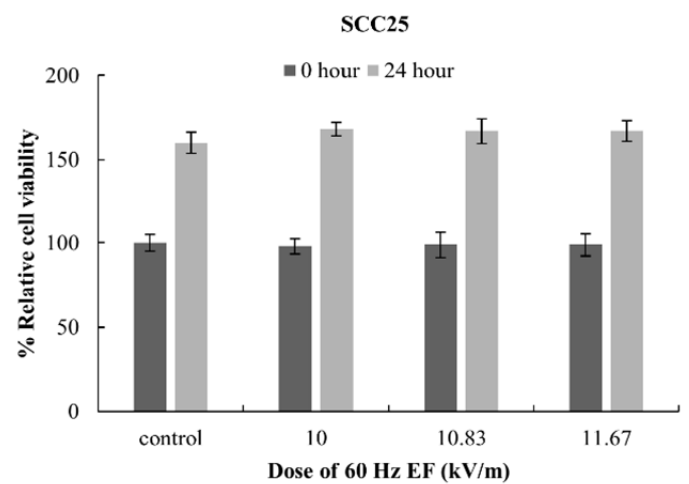### 1. ELF Electric Field Cell Exposure System

To imitate the induced electric fields from the time-varying MF experiment, we designed two types of EF devices. Because the directions of the electric and magnetic fields are perpendicular to one another, the direction of the induced EF was mainly parallel to the surface of a culture dish at the center, where the magnetic field was in a normal direction. However, the induced electric field had perpendicular components as well near the edge of the dish, while the MF had parallel components to the surface.

The first device was composed of two facing electrodes parallel to the dishes, as shown in Fig. 1(a). The powered electrode was located at the bottom surface, and the four grounded electrodes were connected to a sinusoidal voltage source with a frequency of 60 Hz. For safety, the powered electrode was covered with white dielectric material with a relative permittivity of 10 and thickness of 0.18 mm. Four dishes were used at the same time for one experimental set. The maximum electric field in the media was about 10 kV/m. The direction of electric field generated by this device was perpendicular to the dish surface.

To generate electric fields parallel to the dish surface mainly at the center, a coplanar electric field device was designed with two electrodes of 2 cm width and of 12 cm length covered on the bottom side of a plane glass, as shown in Fig. 2(b). The
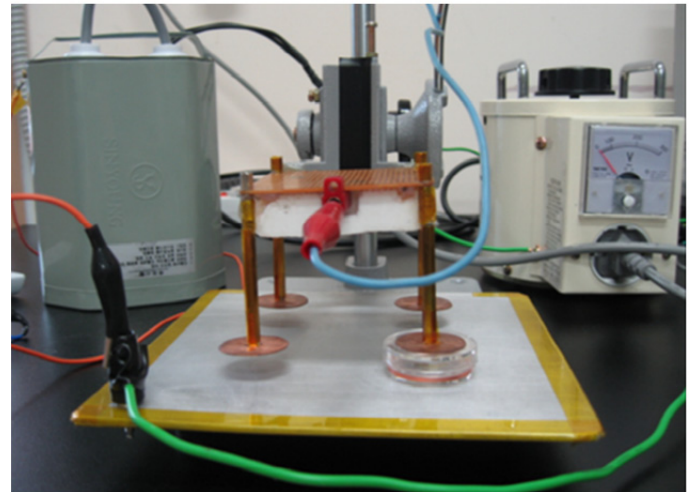
(a)

(b)

Fig. 1. The cellular effect of a perpendicular 60 Hz electric field (EF). (a) The device to generate the 60 Hz perpendicular EF for cell exposures, which is composed of two facing electrodes. (b) SCC25 cells experienced a single exposure to an EF for 30 minutes and were harvested or further incubated for 24 hours. The relative cell viability was determined by MTT assay for each group.

left-side electrode was powered by a bipolar voltage source that had a waveform, shown in Fig. 2(c), and the right-side electrode was grounded. The gap distance was 1 cm. The thickness and the relative permittivity of the glass were 3 mm and 4.2, respectively. For safety, the electrodes were also covered with white dielectric material with a relative permittivity of 10 and a thickness of 0.18 mm. Culture dishes were located on the top of the glass so the center of the dish was at the center of the gap. Three dishes were used for one experimental set. The electrode parts were placed inside an incubator, while the voltage sources were placed outside (Fig. 2(b)). Using a water-jacket incubator, the temperature in the
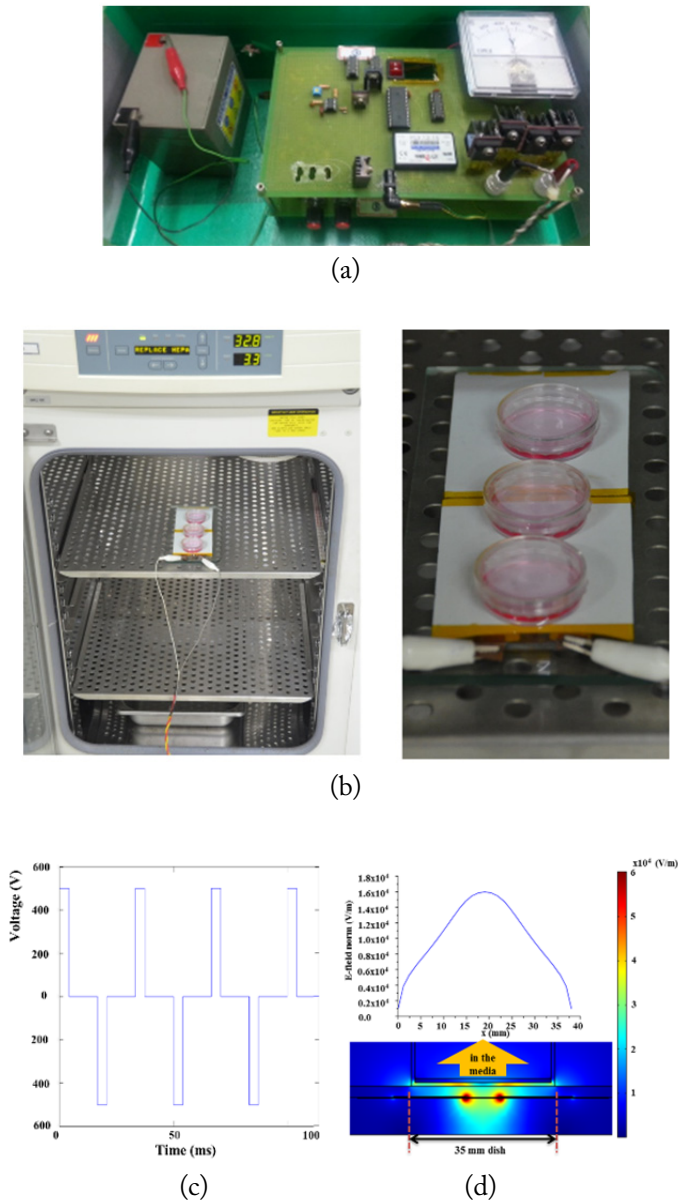
(a)

(b)

(c)   (d)

Fig. 2. The coplanar electric field (EF) device. (a) The coplanar EF generator with two parallel electrodes. (b) The device was placed in the CO_2 incubator (left), and cells were exposed to the coplanar EF in humid CO_2 incubator (right). (c) The voltage waveform with a frequency of 60 Hz and 25% duty ratio was applied to generate an EF intensity of 16 kV/m at the center of the culture dish (left). (d) The computer simulation results for the spatial profiles of the magnitude of the EF intensity for the given voltage waveform (bottom) and the EF intensity along with the bottom surface of the dish (top).

incubator was maintained at 36.7℃–37.1℃ during the experiment.

2. Cell Culture

Human tongue squamous carcinoma (SCC25) and human cervical carcinoma (HeLa) cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco/Invitrogen, Grand

Island, NY, USA), and the primary IMR90 (human lung fibroblast) cells were grown in minimal essential medium (MEM; Gibco/Invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco/Invitrogen) and 10 mL/L penicillin-streptomycin. These cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Both cell types were maintained at 37℃ under a humidified atmosphere of 5% CO_2 and 95% air. HeLa and IMR90 cells were seeded initially at 5×10^4 cells per 35 mm plate for MTT assay and western blotting and were cultured.

3. Exposure to Electric Fields and UV-C

Cells in 35 mm culture dishes were placed on the electrode-containing glass plate and were exposed to the electric fields for different periods of time. For acute response, we exposed a single coplanar EF for 0, 10, and 30 minutes to both HeLa and IMR90 cells. For long-term effects, both HeLa and IMR90 cells were exposed 30 minutes per day or were continuously exposed to a coplanar pulsed electric field (PEF). MTT assays were immediately performed without further incubation. To make p-Chk2 and r-H2AX positive controls, both HeLa and IMR-90 cells were exposed to UV-C radiation for 2 minutes (50 J/m²) followed by incubation in the fresh media for 3 hours.

4. Cell Proliferation Assay

Relative cell viability was assessed by colorimetric measurement of mitochondrial dehydrogenase activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [8]. Cells were immediately incubated with MTT for 1.5 hours or 2 hours after the end of EF exposure. Following incubation with MTT (final concentration 0.5 mg/mL), the produced formazan product was dissolved in 1,000 μL DMSO and determined using a 96-well microplate reader and the software set, SOFTmax PRO 4.0 (Molecular Device Inc., Sunnyvale, CA, USA). Viability results of EF-exposed cells were calculated as the OD_{570} relative percent to that of the EF-unexposed control cells.

5. Western Blot Analysis

To detect phospho-Chk2, poly ADP ribose polymerase (PARP), γ-H2AX, and actin, cells were lysed in 25 mM Tris-Cl buffer (pH 7.6) containing 1% Triton X-100, 1% sodium deoxycholate, 1 mM Na_2EDTA, and 1 mM Na_3VO_4 for 60 minutes at 4℃ and were centrifuged at 14,000×g for 10 minutes. After the supernatants were collected, nuclei in the lysates were collected and histones were extracted with 0.25 M HCl for 30 minutes at 4℃. Protein was quantified by BCA assay. Fifty micrograms of protein were analyzed on a 10% SDS-PAGE, and phospho-Chk2 and PARP were respectively

detected using primary anti-sera of phospho-Chk2 (Cell Signaling Technology, Danvers, MA, USA) and PARP (Cell Signaling Technology). For detection of phospho-H2AX (γ-H2AX), histones were analyzed on a 12% SDS-PAGE and detected with anti-phospho-H2AX (γ-H2AX) primary antibody (Merck Millipore, Billerica, MA, USA). Actin was used as a loading control (Cell Signaling Technology).

III. RESULTS

1. The Effect of Perpendicular Electric Field

We investigated the cellular effect of EFs to understand whether the genotoxic effects of EMFs are mainly due to the electric field generated by an MF. To answer this question, we designed two devices to generate EFs: a 60-Hz sinusoidal EF with two facing electrodes and a 60 Hz bipolar PEF with two coplanar electrodes. The facing electrode device generated EFs perpendicular to the surface of a culture dish, while the coplanar electrode device generated EFs mainly parallel to the surface at the center. The prior MF experiments had induced EFs parallel to the dish surface at the center. Each field generated in the prior MF experiments of them can be connected to a 60-Hz sinusoidal or a 60-Hz bipolar pulsed voltage waveform.

The EF device composed of two facing electrodes parallel to the dishes is shown in Fig. 1(a). The powered electrode was located at the bottom surface, with the four grounded electrodes connected to a sinusoidal voltage source with a frequency of 60 Hz. The EFs by the two facing electrodes shown in Fig. 1(a) were simply calculated as the voltage divided by the gap distance if neglecting the edge effect. A sinusoidal 60 Hz voltage of 9 kV was applied to the facing electrode device with a gap distance of 1.2 cm, resulting in a peak EF intensity of 750 kV/m. However, due to the effect of the large permittivity of the media, more than 80 times larger than that of air, the peak EF intensity in the media would be approximately 10 kV/m. For the same reason, the electric field inside of cells should be reduced again, because the dielectric permittivity of the cell membrane is larger than that of the cell inside. Therefore, the EF intensity in the cell is estimated to be hundreds of V/m, or a few V/cm, which is of the same order of magnitude as what was simulated in Kirson et al. [5]. In this case, the direction of the EF was mainly perpendicular to the dish surface, which was not consistent with the MF experiments.

When we exposed the SCC25 cells to the perpendicular EF generated in this device for 30 minutes and incubated them for 24 hours, there was no acute response related to cell viability (Fig. 1(b)).

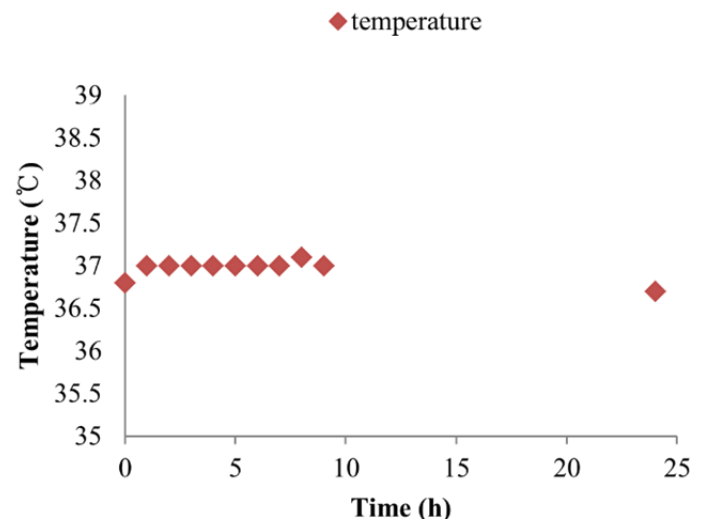### 2. The Parallel Electric Field Applied to the Cell

Fig. 3. The device generating the coplanar electric field (EF) does not increase the inside temperature of incubator. The coplanar EF-generating device was switched on for up to 24 hours, and the temperature in the incubator was monitored at every hour for up to 24 hours.

In order to generate EFs parallel to the dish surface, a coplanar EF device was designed as shown in Fig. 2(a). The device was driven by a pulsed voltage with a frequency of 60 Hz and a duty ratio of 25%, as shown in Fig. 2(c). With this device, a maximum EF intensity of 16 kV/m was achieved at the bottom of the culture dish with media. Higher-order harmonics with frequencies up to hundreds of Hz were generated together, and the effects of several harmonics were included together. However, the magnitude of the high-frequency component was very small for frequencies higher than 1 kHz. The induced MF was negligible, because the rising time and the decreasing time of the pulse was less than 1 ms, during which the biokinetic effect cannot follow. The EFs of this device were calculated using numerical analysis of the Laplace equation, and the magnitude of the EF intensity is shown as a contour plot in Fig. 2(d). The values on the dish surface are plotted together. For the same reason mentioned above, the maximum EF intensity inside the media was much smaller than that of air and was 16 kV/m at the center for the voltage waveform shown in Fig. 2(c). Its spatially averaged value is about 10 kV/m in Fig. 2(d). Contrary to the case of the two facing electrodes, this EF was not uniform, and the direction was mainly parallel to the surface, showing more similarity to the experiments with time-varying MFs. However, the EF intensity induced by the MFs of 60 Hz and 7 mT is an order of mV/m that is much smaller than the EF used in this experiment. When this device for a coplanar EF was put into the incubator, it did not increase the inside temperature of the incubator, indicating that there was no thermal effect of the device on the cells (Fig. 3).

3. A Single Coplanar EF Exposure to HeLa and IMR90 Cells

To assess the effect of a short, single exposure of a-60 Hz coplanar EF to normal and cancer cells, the cancerous HeLa and the primary fibroblast IMR90 human cells were exposed to a 60 Hz, 25% duty ratio and to a 16 kV/m coplanar EF for 10 and 30 minutes with the device described in Fig. 2 and they were compared to the unexposed control. After the exposure of cells to the coplanar EF for 10 or 30 minutes, cell viability was measured by MTT assays [8]. Fig. 4(a) show that neither a single exposure of a coplanar EF for 10 nor for 30 minutes affected the viability of HeLa and IMR90 cells.

Then we investigated whether a single exposure of a 60 Hz coplanar EF would induce any DNA damage in HeLa and IMR90 cells. The most harmful genotoxic stress is the DNA DSB. The biological outcomes of unrepaired DSBs include cell cycle arrest and apoptosis, and incorrectly repaired DSBs lead to carcinogenesis through translocations, inversions, or deletions [9, 10]. The repair of DSBs is more error-prone and may damage the genome integrity, leading to cell death or uncontrolled cell growth, which often exhibits a predisposition towards cancer [11]. One of the earliest cellular responses to DNA DSBs is the phosphorylation of the histone H2AX (yielding γ-H2AX foci) on chromosomes for the recruitment of repair complexes to the site of DNA damage [12-14]. To access DNA damage by a coplanar EF, we examined the phosphorylation of H2AX. We also examined the phosphorylation of Chk2 that is activated to arrest the cell cycle by DNA DSBs. Fig. 4(b) demonstrates that a single exposure of cells to a 60-Hz coplanar EF did not induce any phosphorylation or activation of H2AX and Chk2, while the positive control cells exposed to UV-C (50 J/m^2, 2 minutes) clearly showed p-Chk2 and γ-H2AX (Fig. 4(b)).

4. Repetitive Exposures of HeLa and IMR90 Cells to the Coplanar EF

Since a single coplanar EF exposure of 60 Hz, 16 kV/m did not lead to any changes in cell viability or to DNA damage (Fig. 4), we investigated whether repeated exposures to an EF in the same condition would induce DNA damage. Our previous work showed that repeated exposures to a 60-Hz EMF at 6 mT for 30 minutes every 24 hours induced not only DNA damage but also apoptosis [6]. The repeated exposure to a
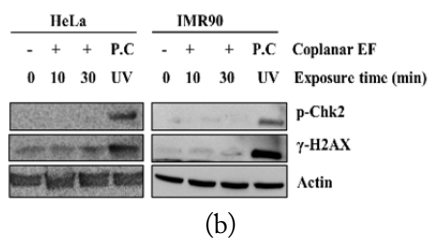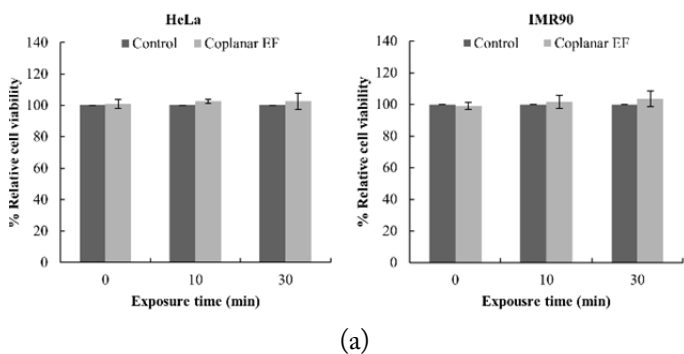

(a)

(b)

Fig. 4. The cellular effect of a single exposure to a 60-Hz coplanar electric field (EF) in the cancerous HeLa and the normal IMR90 cells. HeLa and IMR90 cells were exposed to a single coplanar EF for 0, 10, and 30 minutes, respectively. (a) The relative cell viability of HeLa and IMR90 cells was assessed by MTT assays. (b) Cells were harvested after the exposure to a coplanar EF as described in Materials and Methods section. The expression of p-Chk2 and γ-H2AX was detected by western blots. Actin was used as a loading control. The cells of positive control for DNA damage were produced by UV irradiation as described in Materials and Methods section.
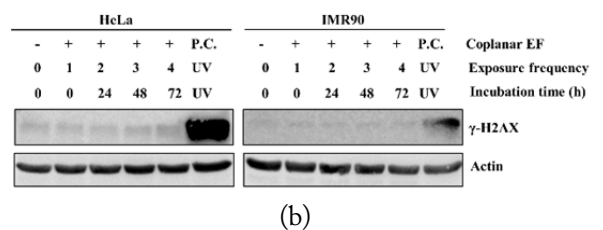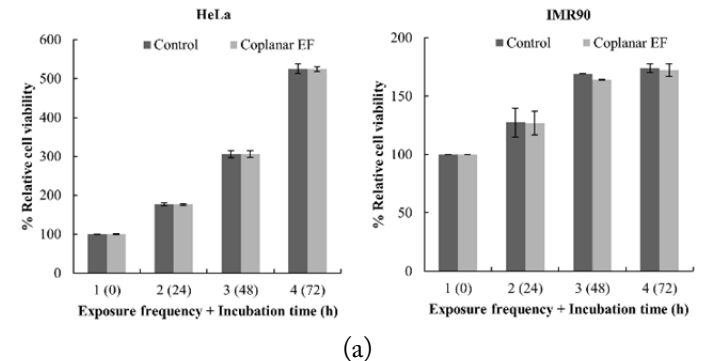

(a)

(b)

Fig. 5. The repetitive exposures of HeLa and IMR90 cells to a 60-Hz coplanar electric field (EF) neither induces DNA damage nor decreases cell viability. HeLa and IMR90 cells were repeatedly exposed to a 60 Hz, 25% duty ratio and to a 16 kV/m coplanar EF for 30 minutes every 24 hours for 3 days. (a) After exposure, the relative cell viability was assessed by MTT colorimetric assays. (b) The cells exposed to a coplanar EF were harvested, and the expression of p-Chk2 and γ-H2AX was detected by western blots. Actin was used as a loading control, and the cells of positive control for DNA damage were produced by UV irradiation as described in Materials and Methods section.

coplanar EF did not show any difference in cell viability compared with the unexposed control, when HeLa and IMR90 cells were exposed to the coplanar EF of 60 Hz for 30 minutes every 24 hours (Fig. 5(a)). In addition, no DNA damage detected in either the HeLa or IMR90 cells after repeated exposures to a coplanar EF (Fig. 5(b)).

5. A Cellular Effect of Continuous Exposure to the Coplanar EF

Thus far, neither single nor repeated exposure to a coplanar EF for a short time induced DNA damage or apoptosis in human cells. Thus, we monitored the effect of a continuous exposure of HeLa and IMR90 cells to the 60 Hz coplanar EF for up to 72 hours. We took the EF-exposed cells every 24 hours and examined viability and DNA damage. As shown in Fig. 6(a), the viability of HeLa and IMR90 cells continuously exposed to a coplanar EF was not changed. Further, we observed neither the phosphorylation of H2AX (r-H2AX) nor the activation PARP that is cleaved downstream of an apoptotic signal (Fig. 6(b)). These observations demonstrate

that continuous exposure to a coplanar EF does not influence on cell viability and DNA damage.

No effect of the continuous exposure to the coplanar EF was further confirmed by extended exposure of the cells already exposed to the coplanar EF for 72 hours. Since cells cannot be maintained in the same medium and dish after 72 hours incubation, each sample of the control and the coplanar EF-exposed cells was treated with trypsin-EDTA to be isolated, and the same number from each sample was subcultured, with further exposure to the 60 Hz coplanar EF for up to another 72 hours. Despite up to 144 hours (72+72) exposure of HeLa and IMR90 cells to a coplanar EF, neither defects in cell proliferation nor DNA DSBs were detected in HeLa and IMR90 cells (Fig. 6(c) and (d)). These results demonstrate that continuous exposure of HeLa and IMR90 cells to a coplanar EF of 60 Hz for up to 72 hours or even up to 144 hours does not affect their viability. No sign of DNA damage or apoptosis was observed in these cells when γ-H2AX and cleaved PARP were detected by western blots.
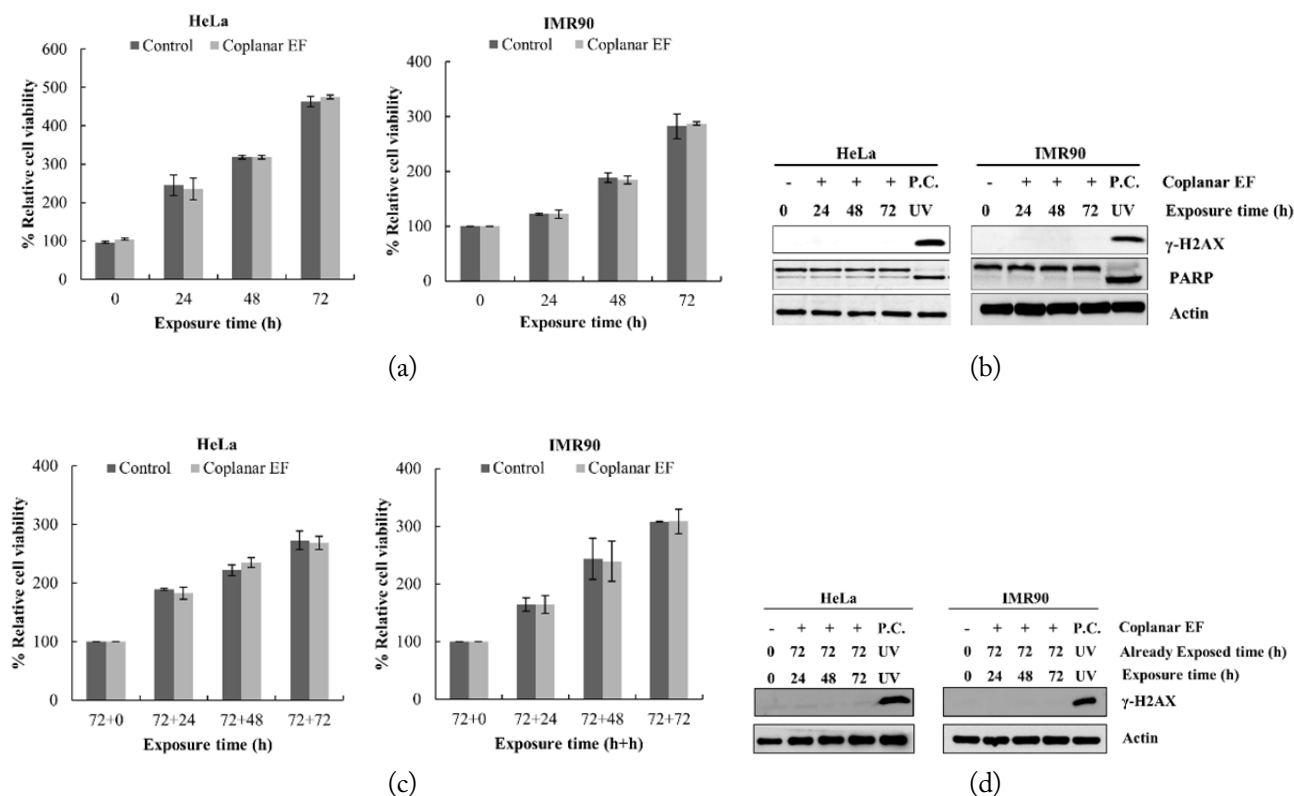

(a)

(b)

(c)

(d)

Fig. 6. The genotoxic effect of the continuous exposure of HeLa and IMR90 cells to a 60-Hz coplanar electric field (EF). (a, b) HeLa and IMR90 cells were continuously exposed to a 60 Hz, 25% duty ratio and to a 16 kV/m coplanar EF for up to 72 hours. After exposure, (a) the relative cell viability was assessed by MTT colorimetric assays, and (b) PARP and γ-H2AX were detected by western blots. (c, d) Cells previously exposed to a coplanar EF for 72 hours were detached by using 0.5% trypsin, seeded in an equal number, and were continuously exposed again to a 60 Hz, 25% duty ratio and to a 16 kV/m coplanar EF for up to another 72 hours. After exposure, (c) the relative cell viability was assessed by MTT colorimetric assays, and (d) PARP and γ-H2AX were detected by western blots. (b, d) Actin was used as a loading control, and the cells of positive control for DNA damage were produced by UV irradiation as described in Materials and Methods section.

Table 1. A guideline for the reference exposure levels of time-varying electric and magnetic fields (unperturbed RMS values) for the general public from the ICNIRP [2]

Range of frequency (f)	E-field strength (kV/m)
1 Hz–8 Hz	5
8 Hz–25 Hz	5
25 Hz–50 Hz	5
50 Hz–400 Hz	$2.5 \times 10^2/f$
400 Hz–3 kHz	$2.5 \times 10^2/f$
3 kHz–10 MHz	8.3×10^{-2}

IV. DISCUSSION

Our previous study revealed that an exposure of HeLa and IMR90 cells to a 60 Hz time-varying magnetic field at 6 to 7 mT for 30 minutes can induce DNA damage [6, 7]. Moreover, repeated exposures to the same time- varying MF for 30 minutes every 24 hours over 3 days can lead to apoptosis and can activate several stress signaling pathways in HeLa and IMR90 cells [6]. However, exposure only to electric fields does not induce DNA damage or cell death, with an EF intensity of tens of kV/m in the media or a few V/cm inside of cells, which is even larger than the EF induced by the time-varying MF in the previous results [6]. Therefore, the genotoxic effect of a time-varying MF on cancer and normal cells is not caused by the induced EFs. Our results suggest that the MF is responsible for the various cellular effects of an EMF. Our observations also showed that neither the direction of a 60-Hz EF nor its waveform affect the cellular response. However, it is worth considering the results of Kirson et al. [4, 5] showing a genotoxic effect of time-varying EFs at hundreds of kHz. Thus, it would be interesting to check the cellular effects of parallel and coplanar EFs in ranges of higher frequencies of kHz for further study.

Our data also showed that there is no cellular effect even when cells are exposed to a higher intensity of EFs than the reference levels of EFs in the safety guideline from ICNIRP (Table 1), suggesting that the guideline for EF exposures may increase the reference EF intensity, since the self-shielding of the body to EFs is much stronger than that of cells [2, 15].

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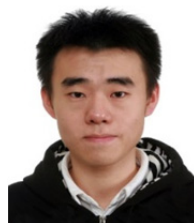
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Gyoo Cheon Kim is a professor in the Department of Oral Anatomy at the School of Dentistry, Pusan National University and a member of the directorial board at the Korean Academy of Oral Anatomy. His research interests include apoptosis in oral cancer cells and plasma medicine for selective cancer cell death, tooth whitening, treatment of oral diseases, wound healing, and skin rejuvenation.