

Effect of Electric Fields on Normal Fibroblast (BJ) Cells

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Abstract: **Background:** Electric fields (EFs) has been reported to affect living tissues. Effects of EFs as anticancer have been recently developed. Designing a therapy that specifically recognize and kills cancer cells without affecting normal cells, has been a recent objective in theranostics. Thus, this study aimed to evaluate the effect of EFs on normal fibroblast cells (BJ cells). **Method:** Cells were incubated for 2, 4, and 6 days with frequency of 50 KHz, 100 KHz, 150 KHz, 200 KHz, 300 KHz. Parameters measured were cells number and viability. Cells number and viability assessment was performed with MTS. **Results:** Number of BJ cells increased each day, compared to 0 day. Number of cells in each day showed no significant difference among treatment of EFs. There was no significant difference of cells viability among treatments each day, and among treatments-0 days, that indicate EFs showed no toxicity toward BJ cells. **Conclusion:** The result of present study showed there was no significant difference among treatment of EFs in normal fibroblast cells. These results indicate EFs did not affect proliferation in normal cell.

Keywords: Electric fields, fibroblast

1. Introduction

Electric fields (EFs) has been reported to affect living tissues. Their activity ranged from stimulating excitable tissues such as nerve, muscle or heart in frequency-dependent manner [Pol, 1995; Palti, 1966], through stimulating bone growth and accelerating fracture healing [Besset, 1985], to using it for diathermy and radiofrequency tumour ablation [Chou, 1995]. Intermediate-frequency EFs (>10 KHz to MHz) were mostly considered as having no biological effect [Elson, 1995] and, hence, medical application, though several non-thermal cellular effects have been observed [Zimmerman et al, 1981; Holzapfel et al, 1982; Pawlowski et al, 1993].

Recently, in vitro and in vivo studies on effects of alternating EFs as anticancer, have been documented [Kirson et al, 2004; Kirson et al, 2007; Zimmerman et al, 2012; Barbault et al, 2009]. These studies showed that anticancer effects were achieved at specific (for the cancer cell type) modulation frequencies and demonstrated proliferative inhibition and mitotic spindle disruption following exposure to alternating electric fields [Kirson et al, 2007; Zimmerman et al 2013]. Designing a therapy that specifically recognize and kills cancer cells without affecting normal cells, has been a recent objective in theranostics [Kievit and Zhang, 2011]. Thus, it is important to observe the effect of EFs on normal cells. In this study, we evaluated effect of EFs on normal fibroblast cells (BJ).

2. Materials And Methods

Cell Culture

BJ cells [ATCC®CRL-2522] were provided by Biomolecular and Biomedical Research Center, Aretha Medika Utama. Cells were grown in α -Minimum Essential Medium Eagle (α -MEM) [Biowest L0475], 10% Fetal Bovine Serum (FBS) [Biowest S181H], 1% Penicillin Streptomycin [Biowest L0022], and maintained at 37°C in humidified atmosphere and 5% CO₂ until the cells were 80-

90% confluence. Growth medium was removed and washed with phosphate buffer saline (PBS) [Gibco 14200075]. Cells were then added with trypsin-EDTA [Biowest L0931-500], incubated at 37°C for 3 min. Trypsinization was stopped by adding growth medium in equal volume. Cells were suspended and replaced into tube, centrifuged at 500 xg for 4 min. Supernatant was removed and pellet were resuspended with 4-5 mL growth medium. Cell suspension was aliquoted into T-flask containing growth medium with density of 8000 cell/cm². Medium was replaced every two days. Cells were incubated at 37°C, 5% CO₂.

Treatments

Cells were washed with 1 mL 1X PBS twice. Cell were then added with 1 mL trypsin EDTA, incubated for 3 min at 37°C, 5% CO₂. Cells were removed to tube containing 5 mL culture medium, and centrifuged at 500 g for 5 min. Supernatant was removed, cells were resuspended with 1 mL culture medium. Cells were plated into 96 wells with 5000 cells each well in 100 μ l medium. Cells were incubated for 2, 4, and 6 days at 37°C, 5% CO₂ with frequency of 50 KHz, 100 KHz, 150 KHz, 200 KHz, 300 KHz. Cells number and viability were measured with 20 μ l (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) [Promega, Madison, WI, USA], and incubated at 37°C for 3 hours. Absorbance was measured using microplate reader (MultiSkan-Go) at 490 nm wavelength.

3. Results

Cell number and viability were evaluated by MTS assay. MTS assay is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays (Malich et al., 1997, Widowati et al., 2013). Absorbance was measured using microplate reader at 490 nm wavelength. Cells number of BJ cells can be seen in Table 1.

Table 1: Effect various incubation periode and various electric frequency toward cells numebr in BJ cells

Treatments	Cells			
	0 day (Untreated)	2 days	4 days	6 days
Control	8,421±397	11,237±549	13,161±1,755	12,650±1,246
50 KHz		11,155±330	12,128±600	11,989±794
100 KHz		11,294±712	11,835±721	11,805±670
150 KHz		11,025±1,280	12,082±948	11,959±1,493
200 KHz		11,596±564	11,209±602	10,947±417
300 KHz		11,949±768	11,376±975	10,937±2,196

Data is presented as mean±standard deviation. Statistical analysis was performed with ANOVA and showed there was no significant difference of cells number among EFs each day.

As shown in Table 1, number of cells in each day showed no significant difference among treatment of EFs.

The BJ cell viability assay was the preliminary study to test cytotoxic of EFstoward BJ cell. Viability was indicated by the conversion of yellow tetrazolium salt to form a purple formazan product (Percentage of viable cells was determined by comparing cells viability value of treatments to the control. BJ cells viability can be seen in Table 2.

Table 2: Viability of BJcellstreated with different EFs in 2, 4, 6 days, and its comparison to 0 day

Treatments	Viability (%)					
	2 days	4 days	6 days	2 days over 0 day	4 days over 0 day	6 days over 0 day
Control	100.00±0.00	100.00±0.00	100.00±0.00	133.64±6.53	156.53±20.87	150.45±14.82
50 KHz	99.37±2.80	93.42±12.73	95.33±8.48	132.66±3.92	144.24 ±7.13	142.59 ±9.45
100 KHz	100.77±8.86	91.38±13.83	94.40±13.99	134.32±8.47	140.76 ±8.57	140.40±7.97
150 KHz	98.13±10.35	93.61±17.47	95.37±15.42	131.12±15.23	143.69 ±11.28	142.23 ±17.76
200 KHz	103.38±6.70	86.32±11.53	87.03±6.17	137.91±6.71	133.31 ±7.16	130.19±4.96
300 KHz	106.57±8.80	87.70±13.08	85.90±12.09	142.11±9.13	135.29 ±11.60	130.07 ±26.12

Data is presented as mean±standard deviation. Statistical analysis was performed with ANOVA, showed there was no significant difference of cells viability among treatments each day, and among treatments-0 days.

As shown in Table 2, there was no significant difference of cells viability among treatments each day, and among treatments-0 days. These results indicate EFs did not affect viability of normal fibroblast cells and considered safe to be further used.

Table 3: Inhibition of BJ cells treated with different EFs in 2, 4, 6 days, and its comparison to 0 day

Treatments	Inhibition					
	2 days	4 days	6 days	2 days over 0 day	4 days over 0 day	6 days over 0 day
Control	0.00±0.00	0.00±0.00	0.00±0.00	-33.64±6.53	-56.53±20.87	-50.45±14.82
50 KHz	0.63±2.80	6.58±12.73	4.67±8.48	-32.66±3.92	-44.24±7.13	-42.59±9.45
100 KHz	-0.77±8.86	8.62±13.83	5.60±13.99	-34.32±8.47	-40.76±8.57	-40.40±7.97
150 KHz	1.87±10.35	6.39±17.47	4.63±15.42	-31.12±15.23	-43.69±11.28	-42.23±17.76
200 KHz	-3.38±6.70	13.68±11.53	12.97±6.17	-37.91±6.71	-33.31±7.16	-30.19±4.96
300 KHz	-6.57±8.80	12.30±13.08	14.10±12.09	-42.11±9.13	-35.29±11.60	-30.07±26.12

Data is presented as mean±standard deviation. Statistical analysis was performed with ANOVA and Tukey post hoc that showed there was no significant difference of inhibition among treatments each day, and among EFs each day.

As shown in Table 3, there was no significant difference of cell inhibition among treatments each day, and among treatments-0 days. These results indicate EFs did not inhibit proliferation of normal fibroblast cells.

4. Discussion

EFs affect intracellular behaviour and interaction among cells. Biological electric plays role in extracelullar and intracellular ion exchange in maintaining cell physiology (McCaig et al, 2005).EFs have antimitotic effects through microtubular spindel breakdown during mitosis, that causes dielectrophoresis dislocation in intracellular molecules and organelles during cytokinesis, resulting in cell membrane

damage and death (Eilon et al, 2004; Eilon et al, 2007).The result of present study showed there was no significant difference among treatment of EFsin normal fibroblast cells.Referring to Polk (1995), alternating EF of intermediate frequencies (10 kHz to 1 MHz) were considered not to have any meaningful nonthermal biological effects.Low frequencies of alternating EFs(under 1 kHz) stimulate excitable tissues through membrane depolarization (Polk, 1995). As the frequency of the EF increases above 1 kHz, the stimulatory effect diminishes. At very high frequencies (*i.e.*, above many MHz), a completely different biological effect is observed. At these frequencies,tissue heating becomes dominant due to dielectric losses. This effect becomes more intense as frequency, field intensity, or tissue dissipation factor increases (Elson, 1995).

In this study, EFs did not affect proliferation in normal fibroblast cell. For our best knowledge, there are only few

studies that report on EFs effect on normal fibroblast cell. Erickson and Nuccitelli (1984) found that fibroblast cells responded to EFs in three ways: (a) the cells migrated towards the cathodal end of the field (b) the cells oriented their long axes perpendicular to the field lines (c) the cells elongated under the influence of field strengths of 400 mV/mm and greater. An increase in calcium translocation and the number of insulin receptors were also documented in human fibroblast in response to an EFs [Bourguignon, 1989], that might be due to opening of voltage-sensitive calcium channels as the primary event by EFs. Moreover, EFs have been reported to generate morphological changes in normal human skin fibroblast with exposure of 0.1 and 0.4 V/mm. Human skin fibroblast react to an external EFs by significant reorientation of their cell bodies, in a perpendicular manner to the EFs [Methot et al, 2001]. However, underlying mechanism of such event remains unclear.

In cancer cells, recent studies showed alternating EFs with intermediate frequency (100-500 KHz) inhibits apoptosis via anti-microtubular in cancer cell growth in breast cancer, lung cancer, and glioma cell line (Eilon et al, 2004; Eilon et al, 2007). However, molecular mechanism of EFs remains unclear. Possible mechanism occurred is via signal transduction (Koga et al, 2012). EFs activate PI3K (*phosphatidylinositol 3-OH-kinase*)-Akt pathway in wound healing process, and p38 MAPK (*Mitogen Activated Protein Kinase*) pathway (Koga et al, 2012; Fukuda et al, 2013). Interestingly, EFs induces p53, enhances transcriptional function of p53, and G2 arrest in cell cycle in human epithelium cells through activation of p21 by p53 that play role as inhibitory protein of Cdk2 (inhibition of synthesis) and Cdc2 (inhibition of mitosis), resulting in G2 arrest (Fukuda et al, 2013).

5. Conclusion

The result of present study showed there was no significant difference among treatment of EFs in normal fibroblast cells. These results indicate EFs did not affect proliferation and untoxic in normal cell.

References

- [1] Barbault A, Costa FP, Bottger B, Munden RF, Bomholt F, Kuster N: Amplitude-modulated electromagnetic fields for the treatment of cancer: discovery of tumor-specific frequencies and assessment of a novel therapeutic approach. *J Exper Clin Cancer Res* 2009, 28:51.
- [2] Besset CA: The development and application of pulsed electromagnetic fields (PEMFs) for ununited fractures and arthrodeseis. *Clin Plast Surg* 1985, 12:259-277.
- [3] Bourguignon GJ, Jy W, Bourguignon LY. Electric stimulation of human fibroblasts causes an increase in Ca²⁺ influx and the exposure of additional insulin receptors. *J Cell Physiol.* 1989;140:379-385.
- [4] Chou CK: Radiofrequency hyperthermia in cancer therapy. In *The Biomedical Engineering Handbook*. Edited by Bronzino JD. Boca Raton, FL: CRC Press; 1995:1424-1430.
- [5] Eilon DK, Dbaly V, Tovarys F, Vymazal J, Soustiel JF, Itzhaki A, et al. Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. *PNAS*; 2007; June 12, .vol. 104 . no. 24. p.10152-10157.
- [6] Eilon DK, Zoya, G, Schneiderman R. Erez Dekel, Aviran Itzhaki, Yoram Wasserman et al. Disruption of Cancer Cell Replication by Alternating Electric Fields. *Cancer Res*; 2004; p.3288-3295.
- [7] Elson E. Biologic effects of radiofrequency and microwave fields: in vivo and in vitro experimental results. In: Bronzino JD, editor. *The biomedical engineering handbook*. Boca Raton, FL: CRC Press, Inc.; 1995. p. 1417-23.
- [8] Erickson, C.A. and Nuccitelli, R., 1984. Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *The Journal of cell biology*, 98(1), pp.296-307.
- [9] Fukuda R, Suico MA, Koyama K, Omachi K, Kai Y, Matsuyama S, et al. Mild Electrical Stimulation at 0.1-ms Pulse Width Induces p53 Protein Phosphorylation and G₂ Arrest in Human Epithelial Cells. *The journal of biological chemistry*; 2013; Vol. 288; NO. 22; p. 16117-16126.
- [10] Holzapfel C, Vienken J, Zimmermann U: Rotation of cells in an alternating electric field: theory and experimental proof. *J Membr Biol* 1982, 67:13-26.
- [11] Kievit FM, Zhang M (2011) Cancer nanotheranostics: improving imaging and therapy by targeted delivery across biological barriers. *Adv Mater* 23(36):H217-H247
- [12] Kirson ED, Dbaly V, Tovarys F, Vymazal J, Soustiel JF, Itzhaki A: Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. *Proc Natl Acad Sci* 2007, 104:10152-10157.
- [13] Kirson ED, Gurchik Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y: Disruption of cancer cell replication by alternating electric fields. *Cancer Res* 2004, 64:3288-3295.
- [14] Koga T, Kai Y, Fukuda R, Morino KS, Suico MA, Koyama K, Sato T, Shuto T. and Kai H. Mild electrical stimulation and heat shock ameliorates progressive proteinuria and renal inflammation in mouse model of Alport syndrome. *PLoS ONE* 7; 2012.
- [15] Lin, Y., Nishimura, R., Nozaki, K., Sasaki, N., Kadosawa, T., Goto, N., Date, M., and Takeuchi, A. (1992). Effects of pulsing electromagnetic fields on the ligament healing in rabbits. *J. Vet. Med. Sci.*, 54, 1017-1022.
- [16] Malich, G. B. 1997. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology*. 124, 179-192.
- [17] McCaig CD, Rajnicek AM, Song B, and Zhao M. Controlling cell behavior electrically: current views and future potential. *Physiol*; 2005; p. 943-978.
- [18] Méthot, S., Moulin, V., Rancourt, D., Bourdages, M., Goulet, D., Plante, M., Auger, F.A. and Germain, L., 2001. Morphological changes of human skin cells exposed to a DC electric field in vitro using a new exposure system. *The Canadian Journal of Chemical Engineering*, 79(4), pp.668-677.

- [19] Palti Y: Stimulation of internal organs by means of externally applied electrodes. *J Appl Physiol* 1966, 21:1619–1623.
- [20] Pawlowski P, Szutowicz I, Marszalek P, Fikus M: Bioelectrorheological model of the cell. 5. Electrodestruction of the cellular membrane in alternating electrical field. *Biophys J* 1993, 65:541–549.
- [21] Polk C, Postow E (1995) *Biological Effects of Electromagnetic Fields Handbooks, Manuals, Etc.* (CRC, Boca Raton, FL), p 618.
- [22] Polk C: Therapeutic applications of low-frequency sinusoidal and pulsed electric and magnetic fields. In *The Biomedical Engineering Handbook*. Edited by Bronzino JD. Boca Raton, FL: CRC Press; 1995:1404–1406.
- [23] Rubin, C.T., Donahue, H.J., Rubin, J.E., and McLeod, K.J. (1993). Optimization of electric field parameters for the control of bone remodeling: Exploitation of an indigenous mechanism for the prevention of osteopenia. *J. Bone Miner. Res.*, 8(Suppl 2), S573–581.
- [24] Zimmerman JW, Jimenez H, Pennison MJ, Brezovich I, Morgan D, Mudry A, Costa FP, Barbault A, Pasche B: Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies. *Chin J Cancer* 2013, 32:573–581.
- [25] Zimmerman JW, Pennison MJ, Brezovich I, Yi N, Yang CT, Ramaker R: Cancer cell proliferation is inhibited by specific modulation frequencies. *Br J Cancer* 2012, 106:307–313.
- [26] Zimmerman U, Vienken J, Piwat G: Rotation of cells in an alternating electric field: the occurrence of a resonance frequency. *Z Naturforsch C* 1981, 36:173–177.

