RF *IN VITRO* EXPOSURE METHODS:
DOSIMETRY AND TEMPERATURE CONTROL

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Introduction

To investigate RF safety, *in vitro* studies on cells or isolated tissues are often desirable. In this presentation, various *in vitro* exposure systems are reviewed. Recording artifacts due to the high conductivity electrodes and the importance of temperature control during *in vitro* experiments are highlighted. Dosimetry involves measurements of temperature or E field in the exposed cell culture and computation by the Finite-Difference Time-Domain (FDTD) method. Engineering evaluation criteria of the IEEE Standard Coordinating Committee 28 Subcommittee 4 are presented to emphasize the important parameters that should be included in an *in vitro* study.

Dosimetry

SAR can be calculated from the electric field or from the temperature rise in tissue. If the temperature rise is used, the SAR is accurately calculated only if no heat diffusion occurs during the RF exposure. There is a misconception that SAR is not useful since it is a thermal unit. SAR is related to the electric field, current density and temperature increment through simple equations. They are all interchangeable. SAR has been adopted world wide for RF dosimetry. For details on RF dosimetry, the readers should refer to a tutorial paper by Chou et al. [1].

In Vitro Exposure Systems

A. Waveguide

Chou and Guy [2] designed an S-band waveguide system for exposing isolated tissues to 2450 MHz microwaves. A quarter wavelength dielectric slab was inserted to match the impedance between the air-filled and solution-filled waveguide. Circulating ports were connected to a constant temperature circulator to minimize microwave thermal effects. Holes on the waveguide walls allowed the isolated tissues to be stretched either parallel or perpendicular to the electric field. This system was used to study pulsed and continuous microwave exposures on the nerve conduction and muscle contraction. No effect other than thermal was observed. In this study a 0.2 ^{o}C temperature rise caused a detectable latency shift in nerve action potential. Temperature control is extremely important in microwave bioeffect studies. Galvin et al. [3] modified the above system to expose two preparations in the same waveguide, using the one away from the dielectric matching slab as a control. This approach was adapted by Liu and Cleary [4] to expose cells. To prevent cells from sinking to the bottom of the culture tubes, magnetic stirrers outside the waveguide was used to keep the cells afloat. However, since the test tube was 8.4 mm ID, it was difficult to keep the temperature constant, especially at high SAR levels. Lin [5] used smaller pipettes for cell culture exposures to minimize the temperature rise. Field et al. [6] used the same waveguide design but with a separately perfused recording chamber to keep the tissue temperature constant. Snail neuron action potentials were recorded with 1 M KCl solution filled microelectrodes. The concentration of the KCl solution was lower than the conventional 3 M solution, because high conductivity solutions can cause artifacts in the microwave field, especially in pulsed microwave fields. The pulsed field can push KCl solution out of the electrode and into cells, which can affect cellular function. Joyner et al. [7] described the design and dosimetry of an automated waveguide exposure system. Czerska et al. [8] exposed human lymphocytes to continuous and pulsed 2450 MHz microwaves in this waveguide system to study the effects on spontaneous lymphoblastoid transformation. The temperature of the cell culture was not controlled. Liburdy and Magin [9] used a tunable 2450 MHz waveguide to expose cell cultures. This device enables continuous control and monitoring of sample temperatures, sample mixing, and pO_2 conditioning. Tuning elements permit absorption of 99% of the forward power. This system was used to study microwave effects on cell membranes.

B. Stripline

Bawin et al. [10] reported the calcium efflux effects using two large triangular aluminum plates ($0.41m^2$) for exposing neonatal chick brains to amplitude modulated (0.5 to 35 Hz) 147 MHz fields. Wachtel et al. [11] exposed Aplysia ganglia to 1.5 GHz microwaves in a stripline to study the microwave effects on the neuron's firing rate. Again, to avoid artifacts due to high conductivity microelectrode fluid, they first filled the electrodes with a 2.5 M KCl solution, then removed all the solution except at the tips, after which they refilled the electrodes with a 0.5 M KCl solution. The temperature of the preparation was not controlled in this experiment. Intensification of E-field at the tip of highly conductive structures depends on the electrode conductivity [12]. Taylor and Ashleman [13] used a parallel plate applicator to expose a cat's spinal cord, which was immersed in a reservoir with perfusing solution to keep the spinal cord temperature constant. Temperature of the spinal cord was measured with a thermistor or a thermocouple, while the microwave exposure was briefly turned off to avoid interference and perturbation. It was difficult to keep the temperature constant because the spinal cord is too large for thermal diffusion to work.

C. Transverse Electromagnetic Cell

Transverse electromagnetic (TEM) cells provide exposure conditions closest to free-space environment and have been used by many investigators for cell culture exposures (e.g., [14]-[16]). In these cells, the electric and magnetic fields are well characterized [17]. The example shown was used for exposing chick forebrains to amplitude modulated RF fields at 50, 147 and 450 MHz in search for the effects on the calcium efflux [18]. The TEM cell was placed vertically and tissue samples were also exposed vertically. The temperature of the samples was controlled by a heater since the samples were exposed at 37 °C instead of at room temperature. Details of another temperature controlled TEM exposure system can be found in Guy et al. [19]. SAR patterns calculated by FDTD method are shown for flasks, petri dishes and test tubes. The FDTD analysis indicated how complicated the SAR dosimetry can be. To keep the cells at a constant temperature is also not easy. Thermal gradient always exists.

D. Coaxial Line

Guy [20] designed a coaxial line system for exposing cells to broadband RF fields (0-100 MHz). The cells were placed inside a 5-ml stainless steel ring with a center conductor and an outer conductor and a Teflon bottom. The electric field orientation is between the inner and outer ring conductor. A Teflon cover kept the cells in a closed space. The culture cup was surrounded by a circulating mineral oil bath to keep the culture temperature constant. The temperature variation of the culture medium, which was monitored by a Vitek temperature sensor, was less than 0.2°C. This result indicated that the mineral oil circulation was able to keep the culture temperature constant. One experiment by pumping the cells to keep them in suspension as compared to the monolayer cells at the bottom if the ring indicated that the importance of thermal gradient. This experiment again signifies the importance of temperature control.

E. Horn Antenna

1) *Near Field:*

Dhahi et al. [21] exposed fungi cells in the near field of an applicator to a continuous wave of 8.7175 GHz. Because the magnetic stirrer was in the near field of the horn antenna, there were standing waves and the field was severely disturbed. The resulting SARs were very complicated. Caddemi et al. [22] placed an isolated chick heart in a petri dish, which was located in the near field of a rectangular slot in a conductor plane and irradiated by an open ended coaxial termination. The micropipets, filled with a low concentration (0.1 M) of NaCl solution, were used to record heart beats. The temperature of the bath was kept at 37°C. Due to the near field configuration, accurate field distribution was difficult to obtain.

2) *Far Field*

To avoid the complicated near-field distribution, *in vitro* cell cultures were exposed in the far field of the antennas. Harrison et al. [23] placed a culture flask in a constant temperature bath, exposed at 20 wavelengths under a horn antenna. A quarter-wave length matching window was placed at the surface of the bath to match the impedance. Field mapping shows that the incident power density was uniform to within 10% in the 25 cm x 25 cm region where the flask was located. With a SAR of 4.4 W/kg at the monolayer cells and at steady state, there was a 1.2 °C temperature rise in the flask. Brown and Marshall [24] exposed murine erythroleukemia cells in cylindrical tubes parallel to the electric field in 1180 MHz far field radiation. Temperature controlled air at 37.4°C was pumped through the exposed region to minimize temperature rise in the test tubes. Dardalhon et al. [25] exposed Chinese hamster cells to 2450 MHz far field microwaves. In addition to circulating cool air, they also rotated the samples to minimize field non-uniformity. Meltz et al. [26] used a styrofoam float in a water bath to hold 10 flasks at the underside and was rotated by water jets. The rotation also averaged the SARs produced by either 915 or 2450 MHz far field exposure. FDTD calculation showed the SAR in a T-25 flask exposed to far field is far from uniform [19].

IEEE SCC28/SC4 Engineering Evaluation Criteria

To revise the IEEE C95.1-1999 RF safety standard, an extensive literature review is underway. The following criteria are being used to evaluate the engineering quality of the papers in the database. A) Exposure parameters: field characteristics, polarization, source of radiation, radiation characteristics, and exposure duration. B) Exposure measurement, C) SAR, induced current and E field in tissue. D) Temperature reporting: temperature in sample, method used (RF non-perturbing probes).

Conclusions

1. SAR of cells exposed *in vitro* varies with dielectric properties and depth of medium; size, shape and orientation of flasks, and the type of exposure systems.
2. Artifacts are hard to recognize and eliminate.
3. Temperature gradient is difficult to detect.
4. Temperature control is essential.
5. Reporting RF field effect must consider all possible confounding factors

References:

1. Chou C.K., et al. Radio Frequency Electromagnetic Exposure: A Tutorial Review on Experimental Dosimetry. Bioelectromagnetics 17(3):195-208, 1996.
2. Chou C.K., and Guy A.W. Effects of electromagnetic fields on isolated nerve and muscle preparations. IEEE Trans. MTT-26:141-147, 1978.
3. Galvin M.J., Parks D.L., and McRee D.I. Microwave irradiation and in vitro release of enzymes from hepatic lysosomes. Radiat. Environ. Biophys. 18:129-136, 1980.
4. Liu L-M, Cleary S. F. Effects of 2.45-GHz microwave and 100-MHz radiofrequency radiation on liposome permeability at the phase transition temperature. Bioelectromagnetics 9: 249-257, 1988.
5. Lin J.C. A new system for investigating nonthermal effect of microwaves on cells. In Johnson C.C. and M.L. Shore (Eds), Biological effects of electromagnetic waves. Selected Papers of the USNC/YRSU Annual Meeting, Boulder, CO, HEW Publication (FDA) 77-8011, II:350-355, 1976.
6. Field A.S., Ginsburg K., and Lin J.C. The effect of pulsed microwaves on passive electrical properties and interspike intervals of snail neurons. Bioelectromagnetics 14:503-520, 1993.
7. Joyner K.H., Davis C.C., Elson E.C., Czerska E.M., and Czerski P. An automated dosimetry system for microwave and thermal exposure of biological samples in vitro. Health Physics 56:303-307, 1989.
8. Czerska E.M., Elson E.C., Davis C.C., Swicord M.L., and Czerski P. Effects of continuous and pulsed 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Bioelectromagnetics 13:247-259, 1992.
9. Liburdy R.P., Magin R.L. Microwave-stimulated drug release from liposomes. Rad. Res.103:266-274, 1985.
10. Bawin S.M., Kaczmarek L.K., and Adey W.R. Effects of modulated VHF fields on the central nervous system. In Tyler P.E. (Ed), Biologic Effects of Nonionizing Radiation. Annals NY Acad Sci, 247:74-81, 1975.
11. Wachtel H., Seaman R., and Joines W. Effects of low-intensity microwaves on isolated neurons. In Tyler P.E. (Ed), Biologic Effects of Nonionizing Radiation. Annals NY Acad Sci, 247:46-62, 1975.
12. NCRP Report 67. RF Electromagnetic Fields; properties, quantities and units, biophysical interaction, and measurements. National Council on Radiation Protection and Measurements, Bethesda, MD., 1981.
13. Taylor E.M., and Ashleman B.T. Some effects of electromagnetic radiation on the brain and spinal cord of cats. In Tyler P.E. (Ed), Biologic Effects of Nonionizing Radiation. Annals NY Acad Sci, 247:63-73, 1975.
14. Blackman C.F., Elder J.A., Weil C.M., Benane S.G., Eichinger D.C., and House D.E. Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effects of modulation frequency and field strength. Radio Sci. 14(6S):93-98, 1979.
15. Sultan M.F., Cain C.A., and Tompkins W.A.F. Immunological effects of amplitude-modulated radio frequency radiation: B Lymphocyte capping. Bioelectromagnetics 4:157-165, 1983.
16. Dutta S.K., Subramoniam A., Ghosh B., and Parshad R. Microwave radiation-induced calcium ion efflux from human neuroblastoma cells in culture. Bioelectromagnetics 5:71-78, 1984.
17. Crawford M.L. Generation of standard EM fields using TEM transmission cells. IEEE Trans. Electromagn. Compat., EMC-16:189-195, 1974.
18. Weil C.M., Spiegel R.J., and Joines W.T. Internal field strength measurements in chick forebrains at 50, 147, and 450 MHz. Bioelectromagnetics 5:293-304, 1984.
19. Guy, A.W., C.K. Chou, J.A. McDougall. A quarter century of in-vitro research: A new look at exposure methods. Bioelectromagnetics 20, Supplement 4: 21-39, 1999.
20. Guy A.W. A method for exposing cell cultures to electromagnetic fields under conditions of controlled temperature and field strength. Radio Sci. 12(6S):87-96, 1977.
21. Dhahi S.J., Habash W.Y., and Al-Hafid H.T. Lack of mutagenic effects on conidia of aspergillus amstelodami irradiated by 8.7175-GHz CW microwaves. J. Microwave Power 17:345- 351, 1982.
22. Caddemi A., Tamburello C.C., Zanforlin L., and Torregrossa M.V. Microwave effects on isolated chick embryo hearts. Bioelectromagnetics 7:359-367, 1986.
23. Harrison G.H., McCulloch D., Balcer-Kubiczek E.K., and Robinson J.E. Far-field 2.45 GHz irradiation system for cellular monolayers in vitro. J. Microwave Power 20:145-151, 1985.
24. Brown R.F., and Marshall S.V. Differentiation of murine erythroleukemic cells during exposure to microwave radiation. Rad. Res. 108:12-22, 1986.
25. Dardalhon M., More C., Averbeck D., and Berteaud A. J. Thermal action of 2.45-GHz microwaves on the cytoplasm of Chinese hamster cells. Bioelectromagnetics 5: 247-261, 1984.
26. Meltz M.L., Eagan P., Harris C.R., and Erwin D.N. Dosimetry considerations in far field microwave exposure of mammalian cells. Physiological Chemistry & Physics & Med NMR 20:23-30, 1988.