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Brief Communications

Optimal Experimental Design for In Vitro Studies With ELF Magnetic Fields

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An experimental arrangement is described that maximizes the dosimetric information that can be obtained during in vitro studies with ELF magnetic fields. The arrangement enables researchers to distinguish between a purely magnetic-field effect and one that also involves the electric fields and currents induced by the magnetic field.

Key words: dosimetry, exposure system, induced current

There have been many laboratory studies since the mid 1970s that have examined the possibility of biological effects in cells in vitro from exposure to extremely low-frequency (ELF) magnetic fields. These studies typically have used Helmholtz coils to generate approximately uniform magnetic fields for exposing the cells. Significantly, the enclosures containing cells and liquid-growth media have varied in geometry and size. Although the magnetic field will be uniform throughout the liquid, regardless of the form and size of the enclosure, the same cannot be said for the electric fields and associated currents that are induced in the liquid as a result of the time-varying magnetic field. In general, the magnitudes (and directions) of the induced currents and electric fields that the cells experience will depend on where the cells are located in the liquid. In addition, the complexity of the equations that predict the values of the induced electric field and current at a point in the liquid will depend on the geometry of the volume of liquid and the direction of the magnetic field.

If, for example, the form of an enclosure containing cells and a liquid medium is approximately box-like, and if the magnetic-field direction is perpendicular to one of the faces of the enclosure, the induced current and electric field have complicated profiles [McLeod et al., 1983]. As a result, cells in the liquid medium can receive greatly differing exposures to the induced electric field or current depending on their

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position. Similar differences will occur if the enclosure is cylindrical and if the direction of the magnetic field is perpendicular to the cylinder's axis. If an attempt is made to confirm the results of an earlier in vitro study emplying ELF magnetic fields, and if there exist differences in the geometry, i.e., differences in form or size of the liquid volume, or in direction of the magnetic field relative to the volume of liquid, the exposure conditions could be significantly different, even if the magnetic field has the same frequency and magnitude. Expressed more simply, a replication of the earlier experiment would not have been performed. Many of the above observations have been made previously or are implied in the paper by McLeod et al. [1983].

The purpose of this brief communication is to recommend a geometry and an enclosure that can be adapted for many in vitro studies employing uniform, single-phase, ELF magnetic fields. (The direction and magnitude of any dc magnetic field, whether ambient or applied, may be of significance if tests of a resonant model are being conducted; such models are not considered here.) The experimental arrangement consists of one or more enclosures, cylindrical in shape, and oriented with the cylinder's axis parallel to the ELF magnetic field. This arrangement can simplify the interpretation of experiments and optimize the amount of information regarding the degree of exposure that cells sustain in the magnetic field, and that is due to the current and electric field induced in the liquid-growth medium by the magnetic field. It is acknowledged, however, because of the multitude of parameters associated with in vitro studies with cells, that it may not be practical in some instances to implement the experimental design being advanced here.

The exposure geometry and the design of an enclosure that would be suitable for short-term studies with cells in suspension are shown in Figure 1. The cylindrical enclosure, which can be made of plastic or glass, has circular walls that partition the cylindrical volume into several sections. As indicated above, the axis of the enclosure is aligned with the magnetic field. Although the cylindrical volume in Figure 1 has been partitioned into three sections, additional or fewer sections could be used. A glass version of the enclosure can be readily fabricated by a glassblower. For more extended periods of exposure, lasting as long as 6 or 7 days, the enclosure can be fitted with a cap and ''sealed'' with commercially available laboratory film, which can prevent significant evaporation but can allow adequate air exchange (Krause, private communication, 1989).

Exposure studies with cells that are cultured in agar may be possible by introducing a layer of agar at the bottom of the enclosure, as shown in Figure 1. If a layer of agar is used, it is desirable that its electrical conductivity be approximately equal to that of the liquid medium so that the two media are electrically equivalent. The agar provides for a more quantifiable exposure of the cells (top and underside) to the induced currents and electric fields as compared with the exposure of cells in contact with glass or plastic.

We note that the use of agar may have unforeseen biological consequences in some cases, as demonstrated by a recent study by Cohen et al. [1988], which showed increased ''. . . frequency of chromosome breakage, sister-chromatid exchange and decreased cloning efficiency. . . .'' in cells cultured in agar as compared with cells grown on a plastic substrate.

If the circular walls shown in Figure 1 introduce an impediment for cells plated directly on the (plastic/glass) enclosure floor, the walls can be replaced with painted concentric circles on the underside of the enclosure floor. Other techniques by which

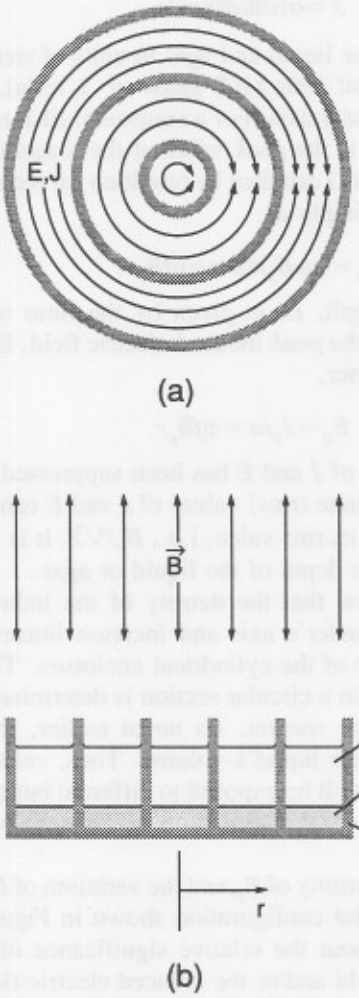

Fig. 1. Geometry of a uniform, magnetic-field cell-enclosure combination: **a:** Top view; directions of E and J are schematically indicated. **b:** Cross-sectional view of enclosure containing agar and liquid medium. The use of agar is discussed in the text. For short-term studies with cells in suspension, circular walls partition the enclosure into regions that provide different exposure conditions.

to characterize the radial positions of the cells in the cylindrical enclosure include growing the cells on a glass cover slip in the enclosure (the cover slip can be removed later for measurements with a compass and rule) or indicating the annular regions on a flat surface on which the enclosure is placed. Vertical stacking of the enclosures is one approach to increase the number of cells that are exposed during an experimental run. The rationale for characterizing the radial positions of cells during exposure to ELF magnetic fields is made clear below.

The directions of the induced current and electric field in the recommended experimental arrangement are circular, as schematically indicated in Figure 1a. The magnitude of the induced current density, J, is given by [McLeod et al., 1983]

$$J = \sigma(dB/dt)(r/2), \tag{1}$$

where σ is the conductivity of the liquid and agar in units of siemans/meter, B is the magnetic flux density in units of tesla (10^4 gauss = 1 tesla), and r is the radial distance in meters from the cylinder axis. For a sinusoidal ELF magnetic field, B can be written as $B_p \sin\omega t$ where B_p is the *peak* value of the magnetic field and ω is 2π times the frequency, f. Equation (1) can then be rewritten for the *peak* current density, J_p, in units of ampere/meter^2, simply as

$$J_p = \sigma\omega B_p r/2 = \sigma\pi f B_p r. \tag{2}$$

Because the electric field strength, E, is given by the ratio of current density to conductivity, the expression for the *peak* induced electric field, E_p, is also simple and is given by, in units of volts/meter,

$$E_p = J_p/\sigma = \pi f B_p r. \tag{3}$$

The sinusoidal time dependence of J and E has been suppressed in Eqs. (2) and (3). It is noted that the root-mean-square (rms) values of J and E can be found from Eqs. (2) and (3) by replacing B_p with its rms value, i.e., $B_p/\sqrt{2}$. It is also noteworthy that J_p and E_p are independent of the depth of the liquid or agar.

Equations (2) and (3) show that the density of the induced current and the electric field vanish on the cylinder's axis and increase linearly to their maximal values along the outer perimeter of the cylindrical enclosure. The range of current-density and electric-field values in a circular section is determined from Eqs. (2) and (3), and the radii that define the section. As noted earlier, the magnetic field is essentially uniform throughout the liquid's volume. Thus, cells placed in different circular regions of the enclosure will be exposed to different but quantifiable levels of electric-field and current density. At the same time, all the cells will be exposed to the same level of magnetic-field.

Because of the spatial uniformity of B_p and the variation of E and J with position in the enclosure, the experimental configuration shown in Figure 1 can be used to investigate directly questions about the relative significance of interaction mechanisms involving the magnetic field and/or the induced electric-field and current densities. For example, the observation of a biological effect at the *same* level in all sections of the enclosure (Fig. 1) would suggest a mechanism involving direct magnetic interaction with the cells, whereas an effect that differed between sections could be indicative of a mechanism involving the induced electric field or the electric and magnetic fields in combination. For the latter case, a single experiment would provide data for a range of electric-field exposures.

In summary, the geometry and design of the cylindrical enclosure are being suggested as a "recommended" experimental configuration because the direction and spatial dependence of B_p, J, and E are readily visualized, the equations for calculating J and E are very simple and independent of liquid depth, the amount of information pertaining to dose-response relations during a single experimental run can be maximized, and the suggested configuration allows one to distinguish between a magnetic-field effect and an effect that involves the electric field or the electric field and the magnetic field in combination. Further, *when possible,* use of a common experimental configuration by researchers would also be helpful when attempts are made to confirm the results of biological studies in different laboratories.

We note that two other groups delivered papers at the 1989 Annual Meeting of the Bioelectromagnetic Society and that these papers are similar in a number of respects to our paper (see Bassen et al. [1989] and Greene et al. [1989]). The material presented here, however, was developed independently of the work by other investigators.

We conclude this communication by urging researchers to measure and report the value of electrical conductivity of their in vitro preparations. (This measurement can be made with commercially available conductivity meters.) There are two reasons why we believe this measurement is important: 1) Conductivity must be known to use Eq. (2) to calculate the current density induced in the preparation by the magnetic field, and 2) knowledge of the preparation's conductivity will be needed to extrapolate results obtained in an in vitro experiment, in which conductivities are in the range of 1 to 2 S/m, to in vivo experiments, in which conductivities can be significantly lower.

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