MORPHOLOGICAL TRANSFORMATIONS OF HUMAN CANCER CELLS AND MICROTUBULES CAUSED BY FREQUENCY SPECIFIC PULSED ELECTRIC FIELDS BROADCAST BY AN ENCLOSED GAS PLASMA ANTENNA

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Abstract

Frequency specific, specially tuned, pulsed, amplitude modulated radio frequency fields utilizing an enclosed gas plasma antenna [Plasma Emission Field Treatment (PEFT)] can have dramatic disruptive effects on the morphology of human cancer cells *in vitro*. The morphological transformations are photographed and video taped.

On the basis of the characterization of the electromagnetic field generated by the gas plasma antenna, a model of the action of the PEFT on the cells, based on the cells microtubule mechanical resonances during their mitotic spindle cleavage, is developed to explain the cells destruction *in vitro*. Furthermore, considering the electric field attenuation inside organs, we can expect a similar destructive effect *in vivo* inside cancerous organs.

Background and objectives

New physical treatments with electric fields applied to cell cultures and to patients suffering from metastatic tumors have recently been published. The anti-proliferation of tumor cells has been greatly enhanced by applying alternating electric fields, designated as Tumor Treating Fields or TTFields, combined with various chemotherapeutic treatments *in vitro* and *in vivo* [1]. The alternating electric fields of low intensity (1-2 V/cm), at frequencies between 100 and 200 kHz, are applied to the skin surface by special insulated electrodes. Despite the use of low intensity electric fields applied to recurrent glioblastoma patients, and the power density between the electrodes maintained below 220 mW/cm^2, this treatment can be considered as a thermal treatment. Another report of experiments with hepatocellular carcinoma cells, *in vivo* and *in vitro,* has been recently published [2] in which a radio carrier frequency of 27.12 MHz is amplitude modulated with a low frequency randomly chosen between 100 Hz and 21 kHz.

There is no known biological mechanism explaining the anti-proliferative effect with such very low intensity electric fields [1] [2], however it appears that the electric field does not affect the division of non-malignant cells and is not responsible for any toxicity, in contrast to the well-established toxicity of most chemotherapy treatments. These authors only suggest that either a specific AC frequency or a specific amplitude modulation frequency is able to destabilize the mitotic spindle during cell division. Because the axis of cell division is randomly oriented, one approach has been to use multiple electric field polarizations sequentially applied to increase the treatment efficacy.

The Plasma Emission Field Treatment (PEFT) utilizes a special plasma antenna, called a "phanotron", which acts according to an isotropic non-polarized emission. The destruction and anti-proliferation caused by the plasma antenna system when applied against cancer cells are obtained by a non-thermal effect. A model based on the microtubule fundamental mechanical resonance frequencies, which are different *in vivo* and *in vitro*, is developed to explain the PEFT results.

Material and Methods

The Rife-Bare Device

The Rife-Bare device (Photo 1) is described in [3]. A digitally synthesized square wave generator (Atelier Robin F125 [www.atelierrobin.net]) in the frequency range of 500 Hz to 250 kHz, duty cycles ranging from 50% to 70%, is used as input to control the amplitude modulation of a 27.12 MHz carrier signal of a prototype radio frequency transmitter (OM2 Plasma Sonics Ltd., Albuquerque, New Mexico) where the index of modulation was higher than 1. This "over modulation" of the carrier frequency results in a pulsed radio frequency output from the transmitter with a complex spectral envelope. The signals are sent through a custom-built 300-W amplifier (Plasma Sonics Ltd.) and then through a special antenna tuner to internal metal electrodes inside the phanotron spherical tube, which is filled with helium gas. The pulsing electric field stimulates the helium atoms into a plasma state. The plasma acts as an isotropic non-polarized antenna emitting a very small non-thermal energy into cells and organs. With a spectrum analyzer coupled to a small circular magnetic loop, we measured the magnetic induction near field B (1) radiated at a modulation frequency f in air medium. We deduced the near electric field E (2).

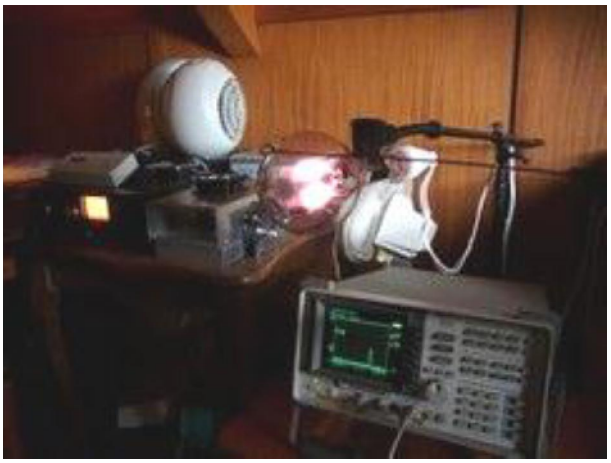


Photo 1: Phanotron Radiation Measurements

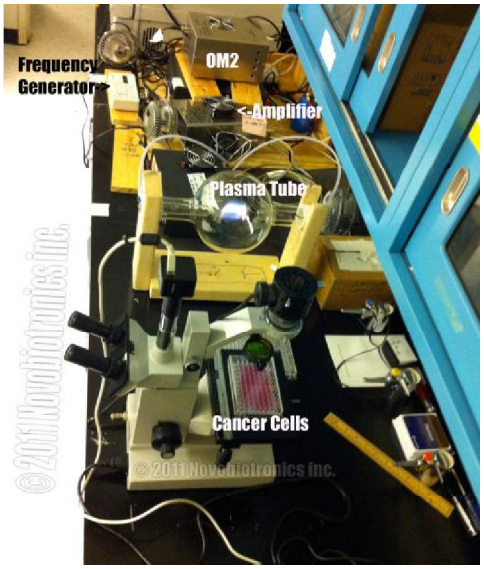


Photo 2: Measurements cells *in vitro*

$$B = \frac{r_0^2}{r^2} \cdot \frac{\sqrt{PZ}}{\sqrt{2}\pi^2 R^2 f} \qquad (1)$$

$$E = \frac{c^2 B}{2\pi f r} \qquad (2)$$

$$P_d = \frac{P}{\pi R^2} \qquad (3)$$

r_0 and r are the radial distances respectively of the loop center and of the observation point from the phanotron spherical plasma tube center. P is the power measured by the spectrum analyzer and P_d is the measured power density at the circular loop level, R being its radius. Z is the standardization resistance and c the light speed in vacuum. B and E respectively follow the law in $1/r^2$ and $1/r^3$.

***Electric field* in vivo**

Inside a living non-magnetic body, the induction magnetic field B is unchanged. On the other hand, the near electric field E_i is equal to (4), $\tilde{n} = n\text{-}jk$ being the living body complex index of refraction:

$$E_i = \frac{E}{|\tilde{n}|^2} \qquad (4) \qquad \text{with } |\tilde{n}|^2 = n^2 + k^2 = \sqrt{\varepsilon_r^2 + \frac{\sigma^2}{\omega^2 \varepsilon_0}} \qquad (5)$$

ε_r and σ are the relative dielectric constant and the conductivity of the living body.

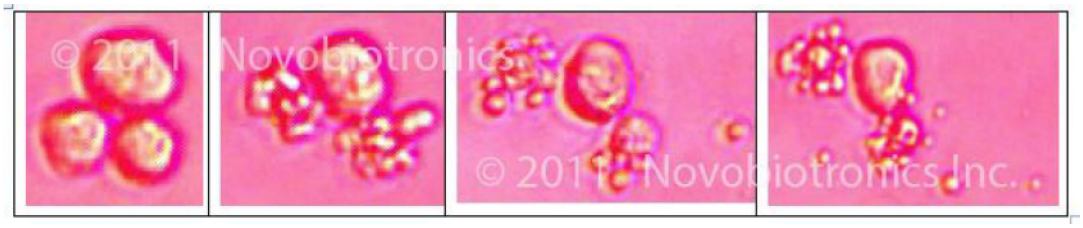
In vitro *experimental set-up*

The plasma tube is located near the stage of an inverted research microscope with a high-resolution video camera (Photo 2). 96-well plates of human cancer cells are set on the microscope stage, exposed to the PEFT and the results are photographed and videotaped in real time. The distance from the phanotron electrodes to the cancer cells is 45 centimeters for all experiments. Pulse rate, pulse envelope shape, power levels and spectral content of the PEFT pulse are correlated with dramatic changes in cancer cell morphology, including fragmentation and total disintegration of many types of cancer cells. Human leukemia and ovarian cancer cells received PEFT alone, while human pancreatic cancer cells received PEFT in combination with low doses of a standard chemotherapeutic agent (Gemcitabine, Eli Lilly and Company).

Results

***Human cancer cell measurements* in vitro**

Several types of human cancer cells were treated with PEFT in experiments (Photo 2) including leukemia cells (K 562 cell line with a modulation frequency of 197 kHz, 10 hours PEFT, 250 W) [4], pancreatic cancer cells (MIA PaCa-2 and Capan-1cell lines: 2 hours PEFT, 150 kHz, 310 W, and 8 hours PEFT: one hour each of 155 to 162 kHz, 310 W) [5], and ovarian cancer cells (A2780 cell line 5 hours PEFT at 114 kHz, 300 W, then 3 hours PEFT at 150 kHz, 325 W [6]. Data from those experiments provide substantial evidence that certain types of cancer cells can be killed and simultaneously slowed in their growth rate using this type of treatment on cancer cells *in vitro*. PEFT resulted in dramatic morphological transformations of a significant percentage of each type of cancer, in many cases resulting in fragmentation and total disintegration of many of the cells. Percent cytotoxicity of PEFT and number of remaining viable leukemia cells treated with PEFT are shown in [3] (Pages 81-84). Cytotoxicity of leukemia cells treated with PEFT is increased by a factor of 3 compared to control cells.



Above: a time-lapse photo montage of three human leukemia cells: two of the cells are shattered into fragments by the PEFT experiment

Above: a time-lapse photo montage of two human pancreatic cancer cells: one of the cells is shattered into fragments by the PEFT experiment

Above: a time-lapse photo montage of human ovarian cancer cells: one cell is shattered into fragments by the PEFT experiment.

Induction magnetic field measurements and deduced electric field in air medium.

Table 1 shows the induction magnetic field B radiated by the phanotron tube measured at a distance $r = r_o = 5$ cm from the sphere center. The modulation frequency range is between 100 and 200 kHz . P(dBm) is the power measured with the spectrum analyzer, B is deduced from (1) with $Z = 70$ ohms, $R = 6\ 10^{-3}$ m and the power density P_d is given by (3).

f (kHz)	100	120	140	150	170	200
P (dBm)	-67	-66	-66	-66	-65	-64
B (T) (1)	$2.4\ 10^{-6}$	$2.2\ 10^{-6}$	$1.9\ 10^{-6}$	$1.8\ 10^{-6}$	$1.7\ 10^{-6}$	$1.7\ 10^{-6}$
P_d (mW/cm^2) (3)	$1.8\ 10^{-7}$	$2.2\ 10^{-7}$	$2.2\ 10^{-7}$	$2.2\ 10^{-7}$	$2.8\ 10^{-7}$	$3.5\ 10^{-7}$

Table 1: Phanotron radiation ($r = r_o = 5$ cm)

The phanotron radiation is isotropic and non-polarized, that is the measured power P is about constant to within 0.5 dB whatever the loop position and its location at r constant around the spherical plasma tube (Photos 1). Such a radiation is due to a set of dipoles randomly distributed between the two electrodes inside a volume of some cubic centimeters. This is an advantage, knowing that the axis of the cell division is randomly oriented during the mitotic spindle cleavage.

As shown in Table 2, the measured induction field B varies following the law $1/r^2$:

r (cm)	5.0	7.2	10.5
Experiments (dB)	0	-6.5	-12.3
Theory (dB) (1)	0	-6.33	-12.89

Table 2: B and its law in $1/r^2$

Tables 3 and 4 below give the radiated electric field E in air medium deduced from B in formula (2).

f (kHz)	100	120	140	150	170	200
E (V/m) (2)	$6.7\ 10^6$	$5.3\ 10^6$	$3.9\ 10^6$	$3.4\ 10^6$	$2.9\ 10^6$	$2.4\ 10^6$

Table 3: Electric field in terms of the frequency radiated in air medium at r = 5 cm

f (kHz)	100	120	140	150	170	200
E (V/m) (2)	$9.2\ 10^3$	$7.2\ 10^3$	$5.3\ 10^3$	$4.6\ 10^3$	$4.0\ 10^3$	$3.3\ 10^3$

Table 4: Electric field in terms of the frequency radiated in air medium at r = 45 cm

The electric field modulus is sufficient to destroy cancerous cells *in vitro* (Table 4: between 92 to 33 V/cm) when, according [1] and [2], a value between 1 and 2 V/cm can arrest cell proliferation in tumors.

Discussion

Microtubule mechanical resonance model

Microtubules play a fundamental role throughout the whole life of eukaryotic cells. For several decades, the dynamic behavior of microtubules has been a subject of great interest. The recent understanding of the microtubule cytoskeleton and the interactions existing among the tubulin subunits and the global static electrical state have been used to propose a special microtubule mechanical model [3] to explain its instability and destruction when the prometaphase of cellular division takes place.

The opposed longitudinal static forces F_S applied at the two ends of one microtubule belonging to the mitotic spindle are given by ([3] p.138) and recalled in (6):

$$F_S = \frac{2Q^2}{\pi\varepsilon_0\varepsilon_r L^2} \qquad (6).$$

L is the microtubule length and Q the static positive and negative electric charges located at its two ends with $Q = 7.5\ 10^{-17}$ Coulombs and $\varepsilon_r = 80$. Then from (6) we have in SI units:

$$F_S = 5\,10^{-24}/L^2 \qquad (7).$$

The static electric field along the microtubule is equal to (8):

$$E_S = F_S/Q = 6.7\,10^{-8}/L^2 \qquad (8).$$

The microtubule is modeled as a hollow cylindrical rod of exterior diameter D and with a thickness of $2R_P$ firmly attached at each of its two ends. The static pressure applied on the microtubule section S is equal to: $P_S = F_S/S$. The protein radius is R_P equal to 2.5 nm.

With (7) and D = 24 nm, we obtain: $P_S = 1.1\,10^{-8}/L^2$ (9) in SI units.

The fundamental static mechanical vibration frequency is equal to:

$$f_r = \frac{3.56}{L^2}\sqrt{\frac{E_L}{\rho}.\frac{I}{S}} \qquad (10).$$

E_L is the Young modulus and $\sqrt{I/S}$ is the microtubule rod gyration radius. For a hollow rod we have: $\sqrt{I/S} = 0.35D$ for $D >> R_P$. Finally with the density $\rho = 10^3$ kg/m^3 we find:

$$f_r = 9.5\,10^{-10}\sqrt{E_L}/L^2 \quad (11).$$

The cell microtubules mechanical instabilities

During the mitotic spindle cleavage, we attribute the morphological transformations of human cancer cells, exposed to PEFT, to the fundamental mechanical resonance frequencies f_r of their microtubules, which depend on their length and Young modulus (11). The study in [8] shows a significant decrease of the bending modulus with increasing temperature equal to 6 10^7 Pa at 20 °C and 2 10^7 Pa at 37 °C. The bending modulus closely corresponds to the Young modulus E_L when $L >> D$. Table 5 shows the microtubule lengths L in terms of their fundamental mechanical resonance frequencies f_r between 100 and 200 kHz ***in vitro*** (20 °C) and ***in vivo*** (37 °C).

f_r (kHz) (11)	L (μm)	
	in vivo 37 °C $E_L = 2\ 10^7$ Pa	***in vitro*** 20 °C $E_L = 6\ 10^7$ Pa
100	6.5	8.6
120	5.9	7.8
140	5.5	7.2
160	5.1	6.8
180	4.8	6.4
200	4.6	6.1

Table 5: Cell microtubule lengths L (micrometers) in terms of their fundamental mechanical resonance frequencies f_r ***in vivo*** and ***in vitro***

These results are consistent with the experimental data in relation to the optimal TTField frequencies for cells and microtubule size [9]. As an example, cell radiuses of 9 and 5 μm correspond approximately to those of melanoma and glioma respectively at frequencies of 100 and 200 kHz.

The conductivity σ and the relative dielectric constant are given in terms of frequency for several organs [7]. Then from the results shown on Table 3 and with (4), we deduced in the figure below, the electric field E_i expressed in V/cm for the frequencies between 100 and 200 kHz at $r = 5$ cm.

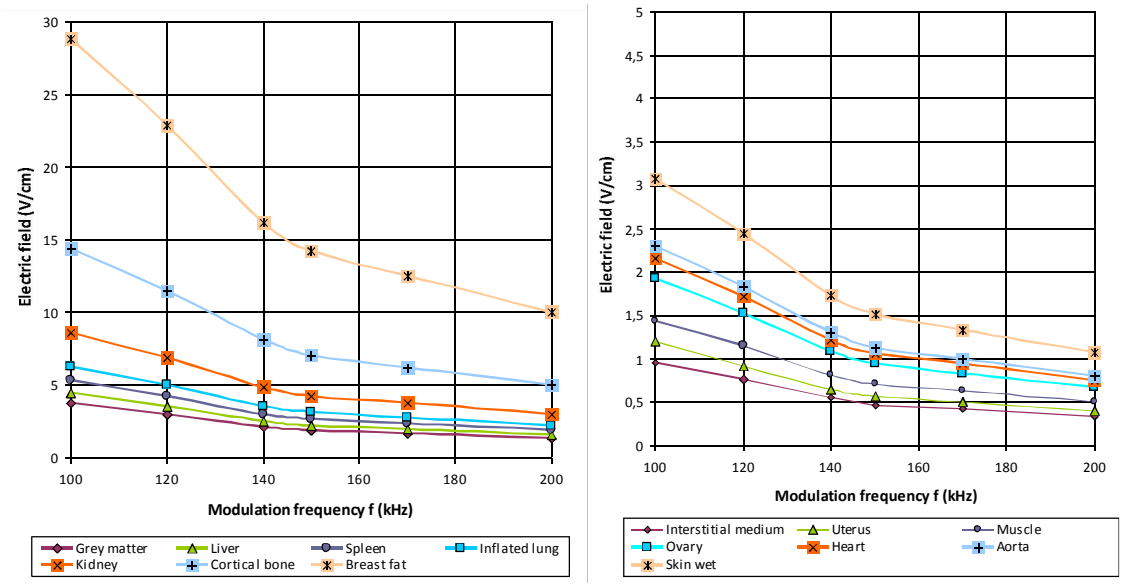


Figure 1: Electric field E_i in various organs in terms of the frequency radiated by a phanotron placed at r = 5 cm

Table 6 below shows r (cm) from the phanotron sphere center for E_i = 1 V/cm at f = 100 kHz.

Organ	***r (cm)***
Uterus	5.30
Muscle	5.65
Ovary	6.21
Heart	6.46
Aorta	6.60
Skin wet	7.27
Grey matter	7.78
Liver	8.22
Spleen	8.72
Inflated lung	9.22
Kidney	10.24
Cortical bone	12.2
Breast fat	15.3

Table 6: Distance of the organs to the Rife-Bare phanotron to expose the organs to an electric field of 1 V/cm

Conclusion

Up to now, there was no known model explaining the anti-proliferative non-thermal effect of very low electric field intensity. The Plasma Emission Field Treatment (PEFT) can have dramatic disruptive non-thermal effects on the morphology of human cancer cells exposed to tumor-specific modulation frequencies ***in vitro*** (temperature = 20 °C) and can be designed to replicate these effects ***in vivo*** (temperature = 37 °C) with a significant lowering of modulation frequencies. The specific carrier wave modulation frequencies are the same as the cancer cell microtubule static mechanical resonance frequencies ($f = f_r$) during the mitotic spindle cleavage. The minimum electric field level of 1 V/cm can be reached by keeping the phanotron close to the subject's body or by increasing the emission power.

In a typical cell culture, the axis of cell division is randomly oriented with only a fraction of the dividing cells subject to optimal treatment by a polarized electric field. Two perpendicular fields were found to be 20% more effective than a single directional field for B16F1 and F-98 cells [10]. Our phanotron electric field emission is perfectly isotropous due to uniformly scattered dipoles between the electrodes located inside the spherical tube.

On the tube surface, the low level measured power density, roughly equal to 10^{-7} W/cm^2, can explain the non-thermal biological effects of PEFT.

Inside different organs, and between 100 and 200 kHz the electric field intensity is on the order of some V/cm.

Electronic cancer treatment devices, other than the Rife-Bare device, which use a fixed frequency applied for a lengthy duration, provide a narrow window of opportunity for effectiveness in slowing and destroying cancer cells. The interaction of the frequency with cell size, local temperature, and spindle length, explains why these other electronic units are not as successful as they should be. Our theory and experimental data also help to explain the reason for slow patient response time with electronic treatments that utilize only one fixed frequency.

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