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PHYSIOLOGICAL AND MOLECULAR GENETIC EFFECTS OF TIME-VARYING ELECTROMAGNETIC FIELDS ON HUMAN NEURONAL CELLS

Thomas J. Goodwin, Ph.D.
Lyndon B. Johnson Space Center

September 2003

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CONTENTS

Figures

CONTENTS (CONTINUED)

Page

Tables

PHYSIOLOGICAL AND MOLECULAR GENETIC EFFECTS OF TIME-VARYING ELECTROMAGNETIC FIELDS ON HUMAN NEURONAL CELLS

Abstract

The present investigation details the development of model systems for growing two- and three-dimensional human neural progenitor cells within a culture medium facilitated by a time-varying electromagnetic field (TVEMF). The cells and culture medium are contained within a two- or three-dimensional culture vessel, and the electromagnetic field is emitted from an electrode or coil. These studies further provide methods to promote neural tissue regeneration by means of culturing the neural cells in either configuration. Grown in two dimensions, neuronal cells extended longitudinally, forming tissue strands extending axially along and within electrodes comprising electrically conductive channels or guides through which a time-varying electrical current was conducted. In the three-dimensional aspect, exposure to TVEMF resulted in the development of three-dimensional aggregates, which emulated organized neural tissues. In both experimental configurations, the proliferation rate of the TVEMF cells was 2.5 to 4.0 times the rate of the non-waveform cells. Each of the experimental embodiments resulted in similar molecular genetic changes regarding the growth potential of the tissues as measured by gene chip analyses, which measured more than 10,000 human genes simultaneously.

Background

Since the 1980s, many methods have been employed in an attempt to accomplish the regeneration of neuronal tissues. Hinkle et al. (1980) detailed the directional growth of frog neurons in an electric field applied by inducing direct DC voltage. Similarly, researchers both nationally and internationally have employed DC electric fields externally and internally as implants to stimulate the direct regeneration of nerve cells in a variety of animal tissues (Borgens et al. 1981,1986, 1986a, 1990, 1997).

These studies have met with limited success, but have shown the potential efficacy of using the inherent characteristics of neural cells to achieve their regeneration. All of these studies incorporate the inclusion of electrodes in direct contact with the tissue of interest.

Other studies have shown that electrically charged polymeric substrates may have value in the stimulation of the regenerative process (Valentini et al., 1992; Makohliso et al., 1992). Additionally, Borgens et al. (1994) substantiates the inference that low-level electric fields and physiological voltage gradients are of importance. However, continuing investigation in these areas has indicated the possibility of a missing component to complete the valuable work of

previous investigations. The missing component may well be the addition of a magnetic element.

Combining the electric and magnetic elements would seem logical, but to date may not have been employed due to traditional purist approaches to experimentation. The current studies are a composite of physiology and electromagnetic bioengineering, which relate generally to the fields of biophysics, tissue regeneration, tissue culture, and neurobiology. The present investigation relates to the use of a time-varying electromagnetic field for potentiation of the growth of mammalian cells and tissues. The preferred protocol uses two-dimensional conducting plate electrodes and may be applied to conventional two-dimensional tissue cultures or to three-dimensional tissue cultures. One may achieve three-dimensional cultures either by exposure to actual microgravity or by using rotating wall vessel (RWV) technology, which simulates some of the physical conditions of microgravity (Goodwin et al., 1993c). The time-varying electromagnetic field is achieved in the vicinity of the electrode by passing a time-varying current pulse with modulated signal through the electrode.

Growth of a variety of both normal and neoplastic mammalian tissues in both mono-culture and co-culture has been established in both batch-fed and perfused RWVs (Schwarz et al., 1991a, 1991b), and in conventional plate or flask-based culture systems. In some applications, growth of three-dimensional tissues in these culture systems has been facilitated by support of a solid matrix of biocompatible polymers and microcarriers. In the case of spheroidal growth, three-dimensional structure has been achieved without matrix support (Goodwin et al., 1992, 1993a, 1993b, 1997). The NASA RWV tissue culture technologies have extended this three-dimensional capacity for a number of tissues and have allowed the tissue to express different genes and biomolecules. Neuronal tissue has been largely refractory, in terms of controlled growth induction and three-dimensional organization, under conventional culture conditions. Actual microgravity, and to a lesser extent, rotationally simulated microgravity, have permitted some enhanced nerve growth (Lelkes and Unsworth, 1997). Previous attempts to electrically stimulate growth have used static electric fields, static magnetic fields, and the direct passage of current through the culture medium, but not the induction of an AC time-varying electromagnetic field in the culture region.

Neuronal tissue comprises elongated nerve cells composed of elongated axons, dendrites, and nuclear areas. Axons and dendrites are chiefly responsible for transmission of neural signals over distance. Longitudinal cell orientation is critical for proper tissue formation and function. The nucleus plays the typical role of directing nucleic acid synthesis for the control of cellular metabolic function, including growth. In vivo, the neuronal tissue is invariably spatially associated with a system of feeder, or glial, cells. This three-dimensional spatial arrangement has not been reproduced by conventional in vitro culture.

Investigators (Borgens et al., Valentini et al., and others) have used static electric fields in an attempt to enhance nerve growth in culture with some success to either alter embryonic development or achieve isolated nerve axon directional growth, but have not yet achieved actual control mechanisms for potentiation of growth or genetic activity causing growth. Mechanical devices intended to help grow and orient three-dimensional mammalian neuronal tissue are currently available. Fukuda et al. used zones formed between stainless steel shaving blades to orient neuronal cells or axons. Additionally, electrodes charged with electrical potential were employed to enhance axon response. Aebischer described an electrically charged, implantable tubular membrane for use in regenerating severed nerves within the human body.

None of these devices uses channels of cell-attractive material, nor do they apply a time-varying electromagnetic field, a static electrical, or a static magnetic field. Additionally, no use is made of simulated or actual microgravity techniques for pure neuronal or mixed neuronal and feeder cell cultures. Incorporation of an electromagnetic stimulus in conjunction with growing three-dimensional mammalian neuronal tissue in the proximity of, or directly upon the surface of, a current-carrying electrode (which may be bioattractive and directly adherent to the cells) is expected to enhance the proliferative response. Furthermore, the use of a time-varying current to induce a corresponding time-varying electromagnetic field in the vicinity of the growing culture to potentiate or spatially direct cell growth is not part of the prior art.

Materials and Methods

Cells

Normal human neuronal progenitor cells (NHNP) were pooled from three donors to diminish donor-to-donor variations in response. As controls, NHNP were grown in conventional tissue culture following standard cell culturing procedures in tissue culture flasks obtained through Clonetics Corporation, San Diego, California.

Cell Culture Protocols

For two-dimensional culture, GTSF-2 medium with 10% FBS, Ciprofloxacin and Fungizone was used to culture the cells (Goodwin et al., 1993a). 1 X PBS, Collagenase, DNase and trypsin were purchased from Clonetics San Diego, California, and used Corning T-75 flasks (Corning Inc., Corning, New York) for initial cell culture to obtain the appropriate number of cells for each experiment. Briefly, cells to be cultured were enzymatically dissociated with the referenced reagents from T-flasks, washed once with PBS-CMF and assayed for viability by trypan dye exclusion (GIBCO, Grand Island, New York). Cells were grown on 100-mm petri dishes (tissue culture treated to prevent adherence) or grown on the actual electrodes inside the petri dishes. Electrodes were made of platinum and stainless steel. Cell cultures were maintained in a humidified Forma CO_2 incubator (Forma, Inc.) at 37°C at a CO_2 concentration of 6%.

For three-dimensional culture, NHNP cells were prepared as described above and an RWV was sequentially inoculated with 5 mg/ml Cytodex-3 type I collagen-coated microcarriers (Pharmacia) and freshly digested NHNP cells, yielding a cell density of 2.5.x 10^5 cells/ml in a 55-ml vessel. Tissues were cultured for 17 to 21 days or until 3- to 5-mm diameter tissue masses formed.

Generator

A waveform (TVEMF) generator of original design and capability was developed and used to generate the waveform in a strength of 1-6 mA (AC) square wave, 10 Hz variable duty cycle, which was pulse-width modulated **(Goodwin et al. patent cases MSC 22633-1; US Patent, 6,485,963, B1 and MSC 22633-2 Notice to Issue**). NHNP cells were subjected to these extremely low-level magnetic fields (ELF waves) (~10 – 200 mGauss), which are far less than the field strength of the Earth. Figure 1 illustrates the experimental arrangements for two-dimensional cell cultures.

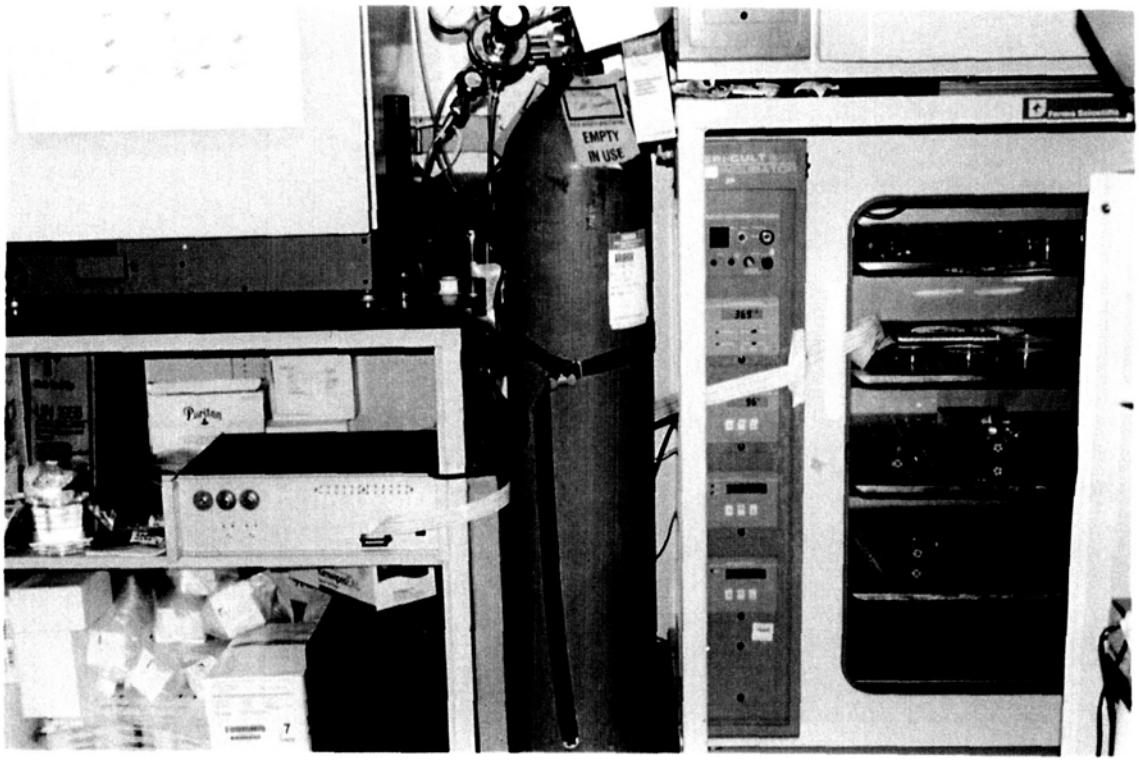

FIGURE 1. Notice the silver-faced box in the left of photo. The generator is attached to the experiment in the incubator by a ribbon cable.

Two-Dimensional Experimental Protocols

Initially, a metal electrode was placed inside a petri dish and centered. NHNP were seeded at 2.5×105 cells in 0.7 ml of media and carefully dropped on the electrode in a concentrated bubble (Figure 2).

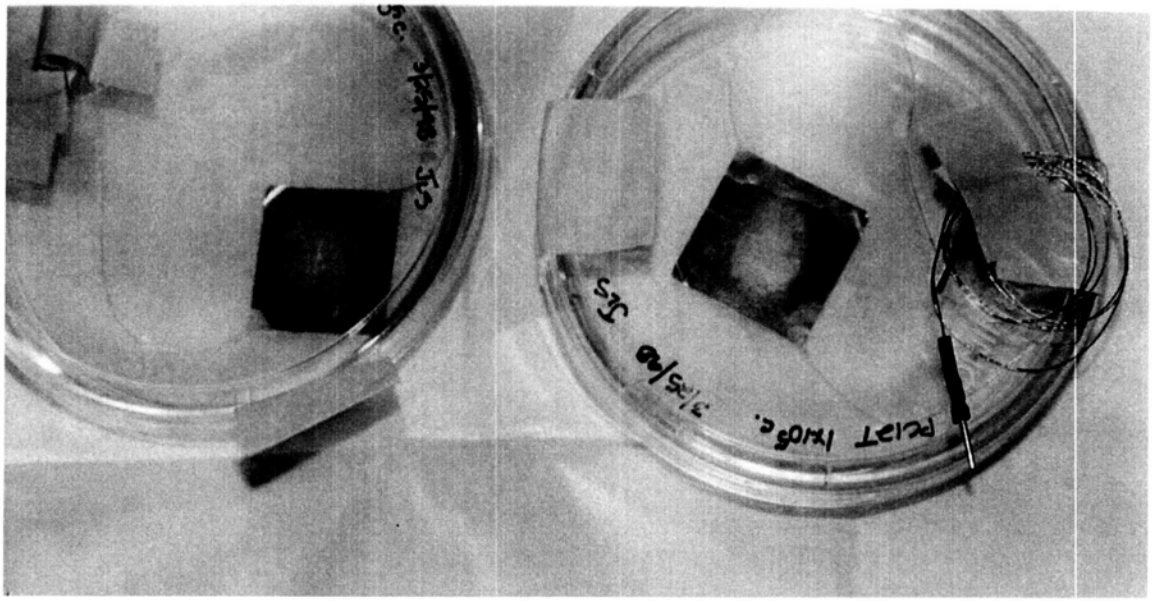

FIGURE 2. The NHNP cells produced a whitish fingerprint on each of the electrodes under TVEMF.

Cells were incubated for 2 days. The second day after cell inoculation is considered day 0 of the experiment protocol. At day 0, each dish was given 15 ml of media and waveform was applied to the electrodes. Cells were fed with 15 ml of media at day 3 and with 13 ml every three days thereafter at day 6, 9, and 12. At days 14 and 17, the cells were fed again with 15 ml of media. At days 17 to 21, the cells were incubated for 10 minutes in a Collagenase/DNase cocktail, then trypsin was directly applied to the cocktail and the cells were further incubated for 3 more minutes. Before the complete media was added to deactivate the trypsin, the cocktail mix was pipetted up and down several times. The cells were washed twice with 1 X PBS, reapplied with the media, and placed on ice. The cells were observed under a dissecting microscope, counted, and assessed for viability.

An identical protocol was followed in similar experiments with the exception that, instead of the electrode being placed within the petri dishes, in media, it was attached to the underside of the TVEMF treated dishes, so that the cells had no direct contact with the metal surface.

Three-Dimensional (RWV) Experimental Protocol

Three-dimensional neural cells and tissues were cultured by the method described above, except that the TVEMF RWV was modified to incorporate an electromagnetic coil. The coil was wrapped around the core of the vessel so it emitted the same electromagnetic field strength as in the two-dimensional configuration. All other conditions were identical to the two-dimensional experimental conditions.

Analytical Methods

Cell Metabolic Parameters

The following metabolic parameters were monitored on a daily basis during the culture of the RWV model: pH, dissolved O_2, dissolved CO_2, glucose concentration, cell viability, and LDH (lactate dehydrogenase). Dissolved O_2, CO_2, pH, and glucose are routinely measured in our laboratories (Goodwin et al., 1992, 1993b; Jessup et al., 1994; Scharz et al., 1992). At 24-hour intervals, pH, CO_2, and O_2 were determined on a Corning 238-model clinical blood gas analyzer (Corning, Corning, New York). The glucose concentration was determined by a Beckman 2 model clinical glucose analyzer (Beckman Instruments, Inc., Fullerton, California). Samples for determination of LDH levels were collected each 24-hour period. LDH analyses were performed on a clinical Kodak Biolyzer (Kodak, Inc., New Haven, Connecticut) using dry chemistry technique.

Light Microscopy

Two-dimensional samples of each experiment were photographed by a Nikon T330 microscope equipped with advanced Hoffman Modulation optics (Nikon Inc., New York, New York).

Scanning Electron Microscopy

Samples for scanning electron microscopy were harvested every 48 hours of culture and were washed once in PBS-CMF, fixed in a solution containing 3% glutaraldehyde, 2% paraformaldehyde in 0.1M cacodylate buffer at pH 7.4, processed by RMC, Inc., and scanned at 10 kV.

Genetic Discovery Array

Samples of human NHNP cells grown in conventional flask culture were trypsinized and placed into petri dishes in a 55-ml RWV. After 17-21 days of culture on 5 mg/ml cytodex-3 beads, cells were washed once with ice-cold phosphate buffered saline. The cells were lysed and mRNA selected with biotinylated oligo (dT) then separated with streptavidin paramagnetic particles (Poly A Ttract System 1000, Promega Madison, WI). 32P-labeled cDNA probes were generated by reverse transcription with 32P dCTP. The cDNA probes were hybridized to identical gene discovery array filters (Incyte Genome Systems Inc., St. Louis, Missouri). The gene discovery

array filters contain over 12,000 unique human genes from the I.M.A.G.E. Consortium (LLNL)1 cDNA Libraries, which are robotically arrayed on each of a pair of filter membranes. Gene expression were then detected by phosphor imaging and analyzed using the Gene Discovery Software (Incyte Genome Systems Inc., St. Louis, Missouri).

Statistical Analysis

All experimental and control groups were analyzed in triplicate or quadruplicate, depending on the availability of materials. These were averaged for determination of mean and standard error. Frequencies in phenotypic analysis were compared by chi-square. Proliferation as assessed by 3H-TdR uptake was compared by analysis of variance for multiple groups, using the Student's *t*-test for paired groups (Zar, 1974).

Results

Two-Dimensional Experiments

NHNP pool cells exposed to a TVEMF, whether or not in direct contact with the electrode, displayed an accelerated growth rate and different morphology as compared to non-waveform cells (controls) (Tables 1 and 2).

TABLE 1. Average Experimental Data.
Cells were in direct contact with the electrode.
Viability 98%, Harvest 17 days.

NHNP-POOL	CELL COUNT
TVEMF 1	860,000
TVEMF 2	1,000,000
TVEMF 3	1,000,000
TVEMF 4	1,300,000
TVEMF 5	1,000,000
TVEMF 6	940,000
TVEMF 7	700,000
TVEMF 8	1,000,000
Control 1	500,000
Control 2	400,000
Control 3	300,000
Control 4	500,000
Control 5	400,000

After the application of the TVEMF, the cells preferentially aligned, while cells without waveform exposure showed a normal random pattern. Cells in direct contact with the electrode remained stimulated up to 72 hours after the TVEMF was removed (Table 3), while those not in direct contact with the electrode continued to experience an accelerated and long-term stimulation in growth pattern even 168 hours after the TVEMF was removed (Table 4). Viability was also higher in the cells exposed to the waveform (Table 4). In each case, cell growth returned to normal rates without further TVEMF stimulation.

To examine the accelerated growth pattern, called the *Corona Effect*, after treatment with TVEMF, the same treatment was applied to the cells without harvesting. Several petri dishes, each from the TVEMF group and non-waveform groups, were chosen randomly. Cells still attached in sheet form were lifted off of the electrodes carefully and placed in new petri dishes with medium, and then photograph 24 hours later (Figs. 4-6).

TABLE 2. Average Experimental Data.
Cells were not in direct contact with the electrode.
Viability 100%, harvest 18 days.

NHNP-POOL	CELL COUNT
TVEMF 1	1,000,000
TVEMF 5	1,100,000
Control 1	800,000
Control 5	800,000

TABLE 3. Average Experimental Data.
Cells were in direct contact with the electrode.
Cell count at various times after removal of TVEMF.

CELLS	COUNT	HOURS OFF ELECTRODE
TVEMF 11	520,000	Counted and re-seeded at 96,000/plate
Control 6	112,000	Counted and re-seeded at 96,000/plate
TVEMF11	274,000	48 hours off electrode
Control 6	48,000	48 hours off electrode
TVEMF 12	576,000	Counted and re-seeded at 96,000/plate
Control 7	96,000	Counted and re-seeded at 96,000/plate
TVEMF 12	228,000	72 hours off electrode
Control 7	120,000	72 hours off electrode

Three-Dimensional Experiments

The ability to influence and, to some degree, control, the growth patterns of NHNP cells in the two-dimensional aspect is a great investigational tool. The question that emanated from this result was, did we have the ability to construct a three-dimensional model and have it emulate the same genetic influence of the TVEMF as shown in Figures 4 and 5? To answer this question, we turned to a new technology called RWV.

TABLE 4. Average Experimental Data.
Cells were not in direct contact with the electrode.
Cell count and viability at various times post harvest.

NHNP-POOL	CELL COUNT	VIABILITY	TIME AFTER HAVEST (Hours)
TVEMF 1	56,000	85%	24
TVEMF 5	40,000	85%	24
Control 1	36,000	65%	24
Control 5	28,000	65%	24
TVEMF 1	188,000	98%	48
TVEMF 5	212,000	98%	48
Control 1	74,000	87%	48
Control 5	162,000	90%	48
TVEMF 1	3,400,000	100%	120
TVEMF 5	3,400,000	100%	120
Control 1	900,000	99%	120
Control 5	900,000	99%	120
TVEMF 1	4,000,000	100%	168
TVEMF 5	3,800,000	100%	168
Control 1	980,000	97%	168
Control 5	900,000	95%	168

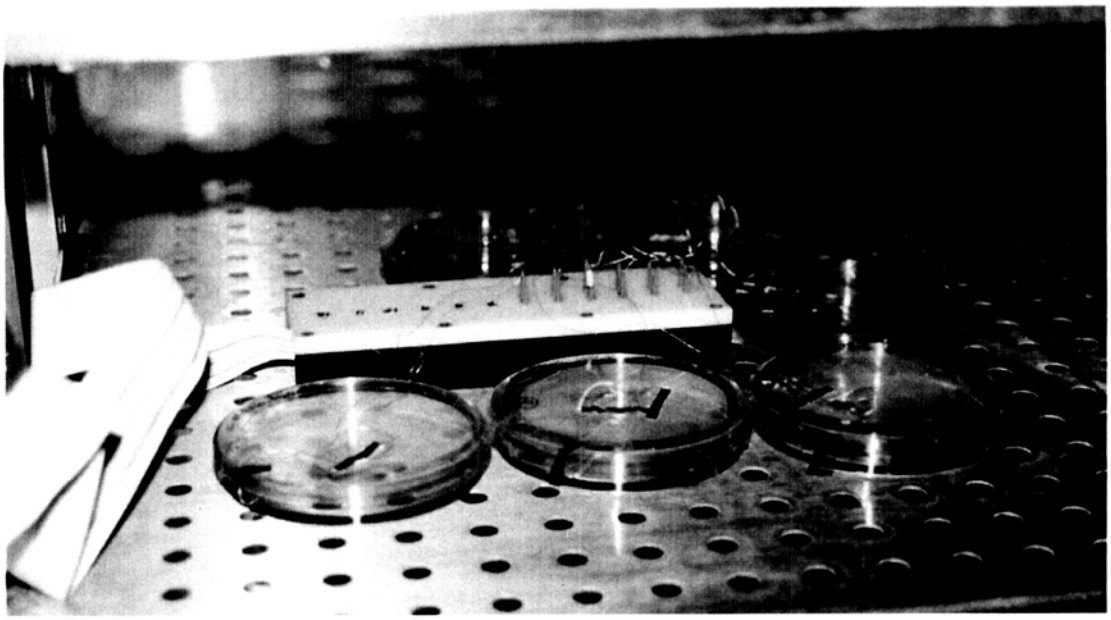

FIGURE 3. NHNP dishes with TVEMF applied.

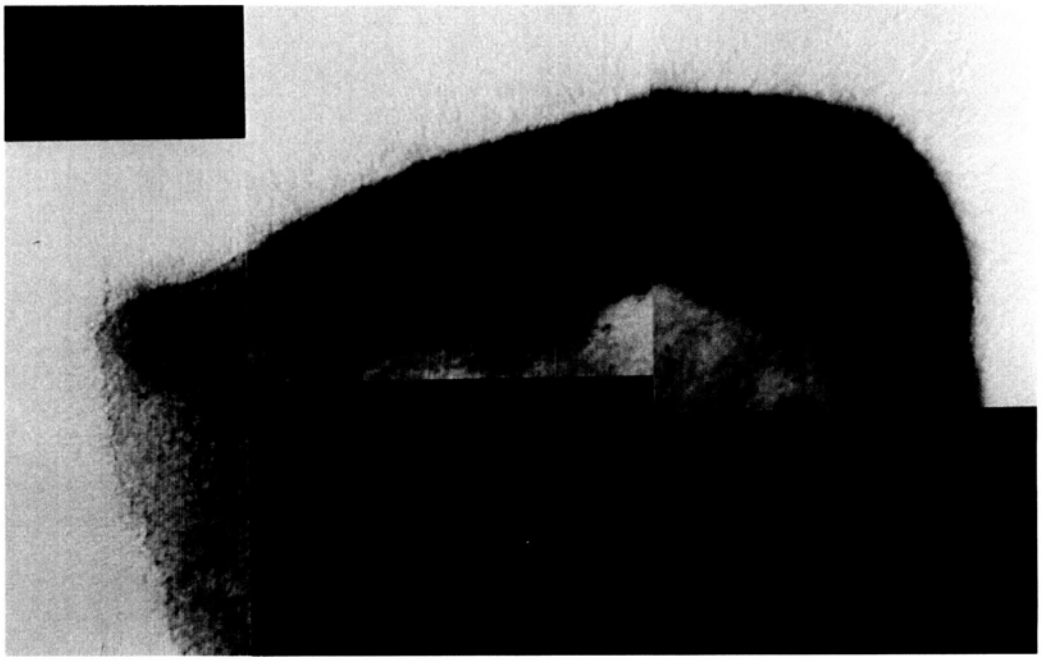

FIGURE 4. A corona can be seen at the edge of the tissue 24 hours after TVEMF.

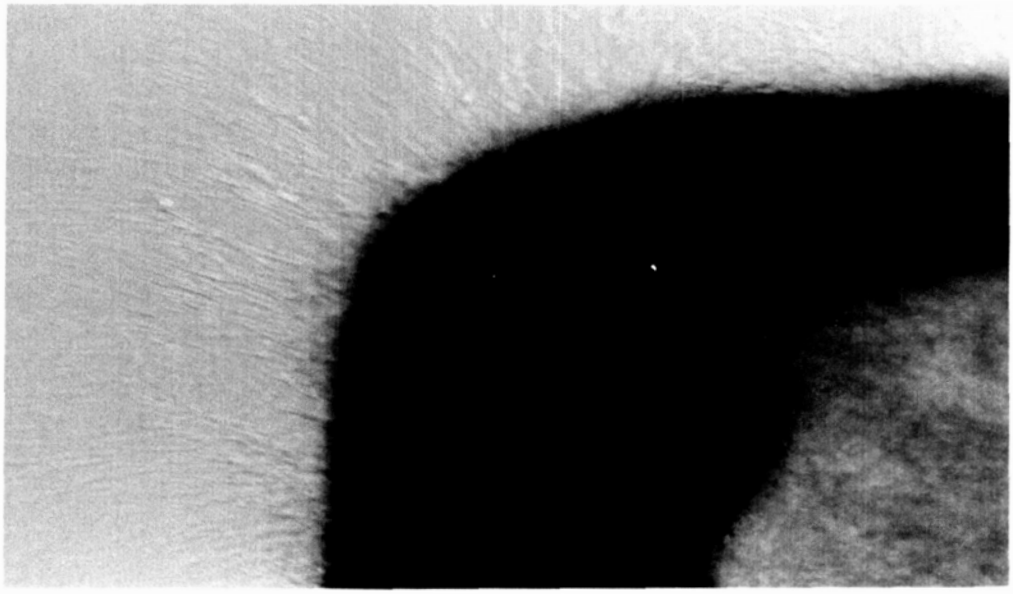

FIGURE 5. Close inspection of the edge of the corona reveals NHNP cells attempting to grow in an oriented fashion away from the transplanted tissue.

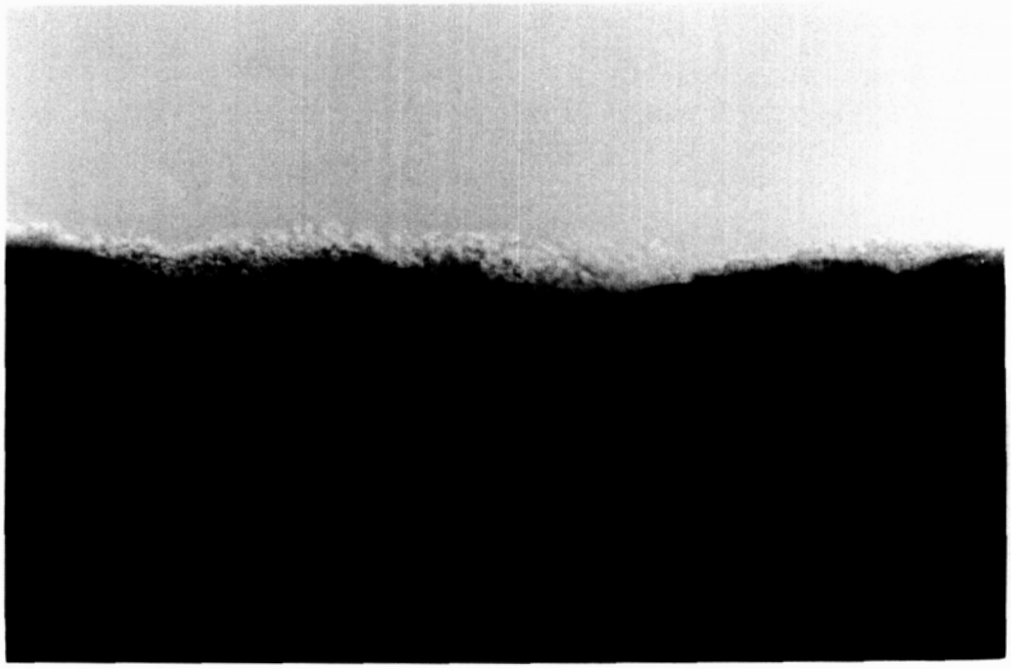

FIGURE 6. In direct contrast to the treated NHNP cells, non-waveform tissues attached but did not proliferate over the same 24-hour period.

Rotating-Wall Vessel Background

The RWV was created at the Johnson Space Center in 1985 to address the problems associated with attempting to simulate microgravity conditions in an Earth-bound lab. It was designed to maximize the conditions of free fall while allowing continuous cell culturing over prolonged periods of time. This instrument uses a cylindrical tube with suspended particles that is rotated inline with the horizontal axis. The particles move in conjunction with the fluid and the wall of the vessel, the effects of gravity are randomized, modeling some aspects of microgravity, while reducing to a minimum shear and turbulence (Dedolph and Dipert, 1971; Cogoli and Gmünder, 1991; Schwarz et al., 1992; Prewett et al., 1993, Goodwin et al., 1993).

Several fluid dynamic operating principles define the RWV. First, it is composed of a solid body rotation about a horizontal axis characterized by (a) co-location of particles of different sedimentation rates, (b) extremely low fluid shear stress and turbulence, and (c) three-dimensional spatial freedom. Second, oxygenation is by active or passive diffusion so that only dissolved gasses are present in the reactor chamber. There are no gas bubbles and no gas/fluid interface (Wolf and Schwarz, 1991; Schwarz et al., 1992; Goodwin et al., 1993).

Rotating-Wall Vessel Experimental Data

We modified an RWV by adding an electromagnetic field coil to allow observation of TVEMF influence on NHNP cells in three-dimensional aspect. Using the RWV, we replicated our preliminary results from the two-dimensional studies several times, and have analyzed the gene expression using gene arrays. We monitored cell proliferation, orientation, morphology, glucose metabolism, and pH (Figs. 7-11). Our results show a stable, reliable model to study the control of high-level cellular processes by application of low-amplitude, time-varying micromagnetic fields.

Figures 7 to 11 show that metabolic parameters evidenced little or no differences between TVEMF and control three-dimensional culture. This would indicate that, although cellular growth was enhanced and more directed, additional cellular energy was not required to accomplish this effect. The RWV-grown tissues also emulated the same basic genetic response as the two-dimensional tissues at similar fields strengths.

This study focused on the use of NHNP cells because of their importance in human nervous system development and maintenance. However, we have developed two-dimensional and now three-dimensional bioreactors that can potentially accommodate other cell lines. Initial results with the NHNP cells were quite startling, using extremely low-level magnetic fields (~10 – 200 mGauss), below the magnetic field strength of the Earth itself (approximately 500 mGauss). We found the low-amplitude, rapidly time-varying magnetic fields exerted a very potent effect on the proliferation, morphology, and gene expression of the cells in culture, both in standard 2-dimensional culture plates (Fig 12) as well as cells organized into 3-dimensional tissue clusters (Fig 8) in the RWV.

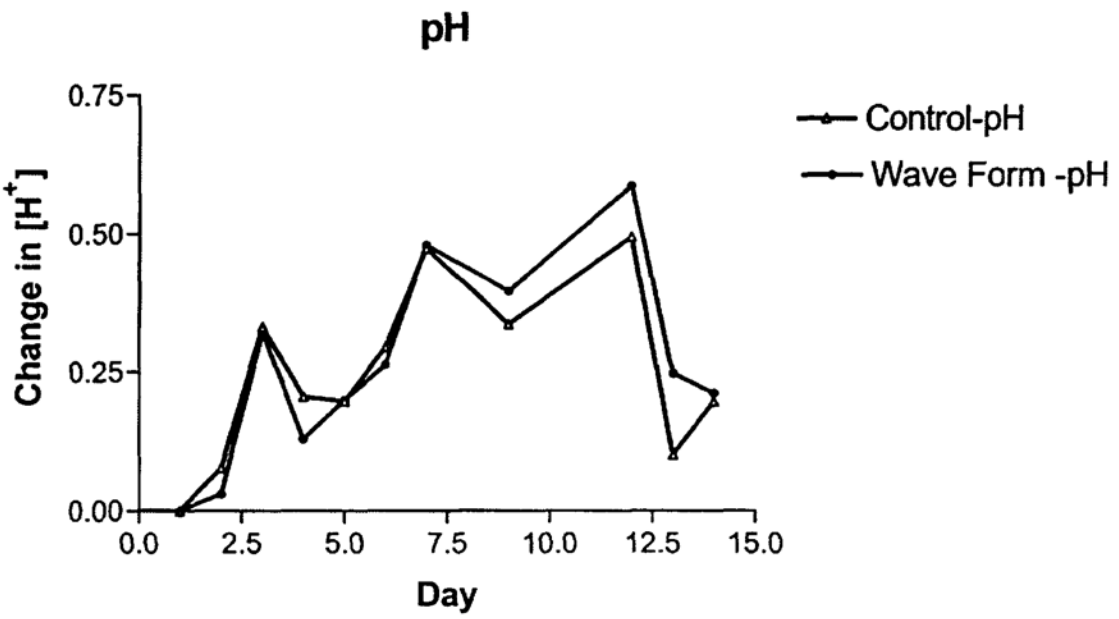

FIGURE 7. Changes in pH between treated and control cultures in RWVs was minimal, indicating the overall metabolism was nearly identical in both vessels.

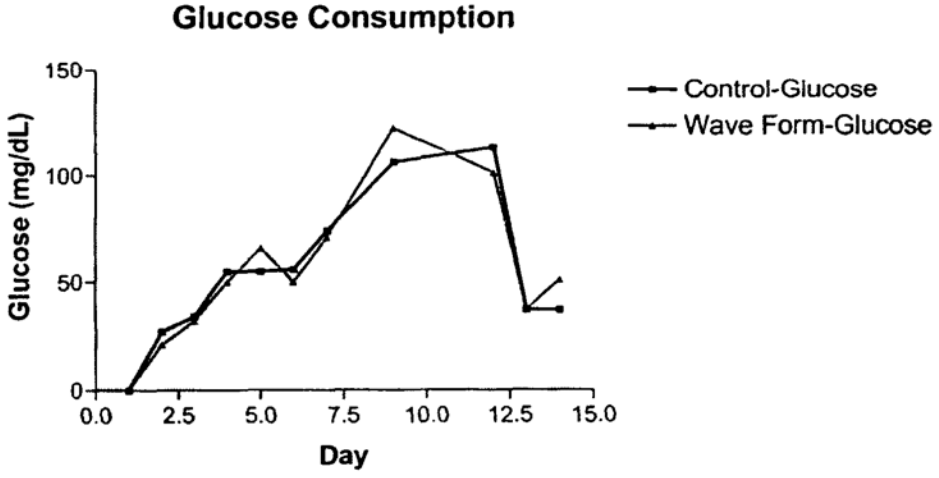

FIGURE 8. Glucose consumption was identical during the course of the experiments.

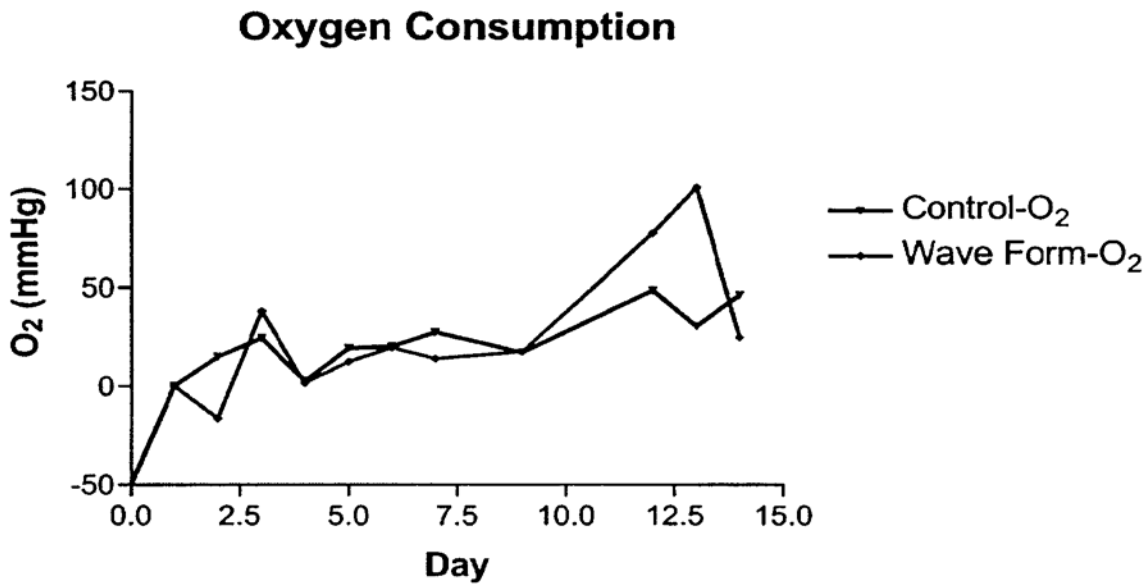

FIGURE 9. Oxygen consumption was slightly higher in TVEMF in the later stages of the experiments.

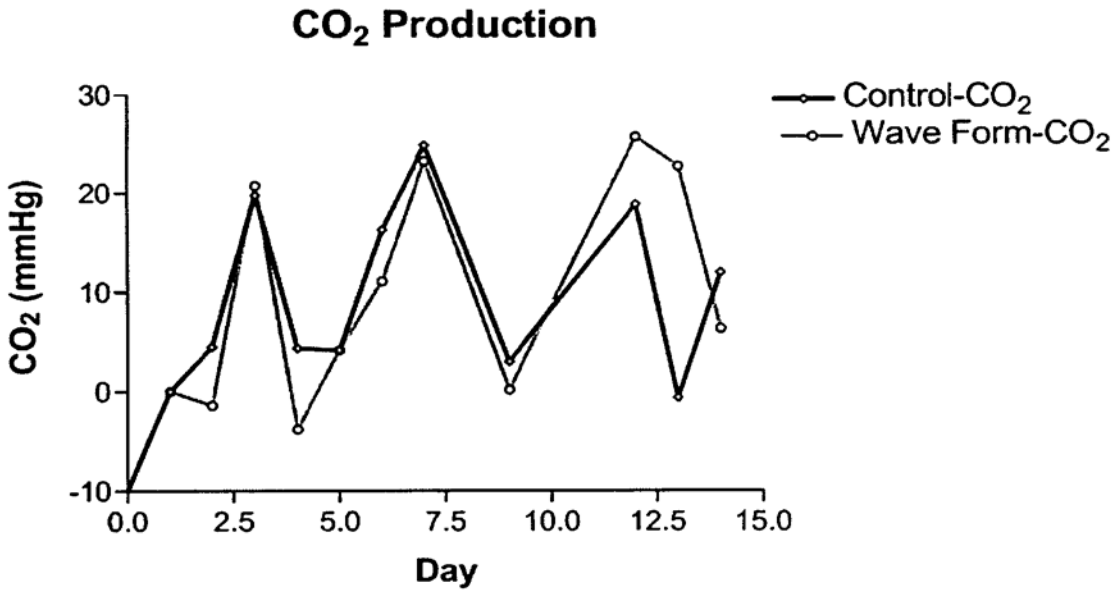

FIGURE 10. As would be expected, TVEMF CO_2 levels were slightly higher than in controls.

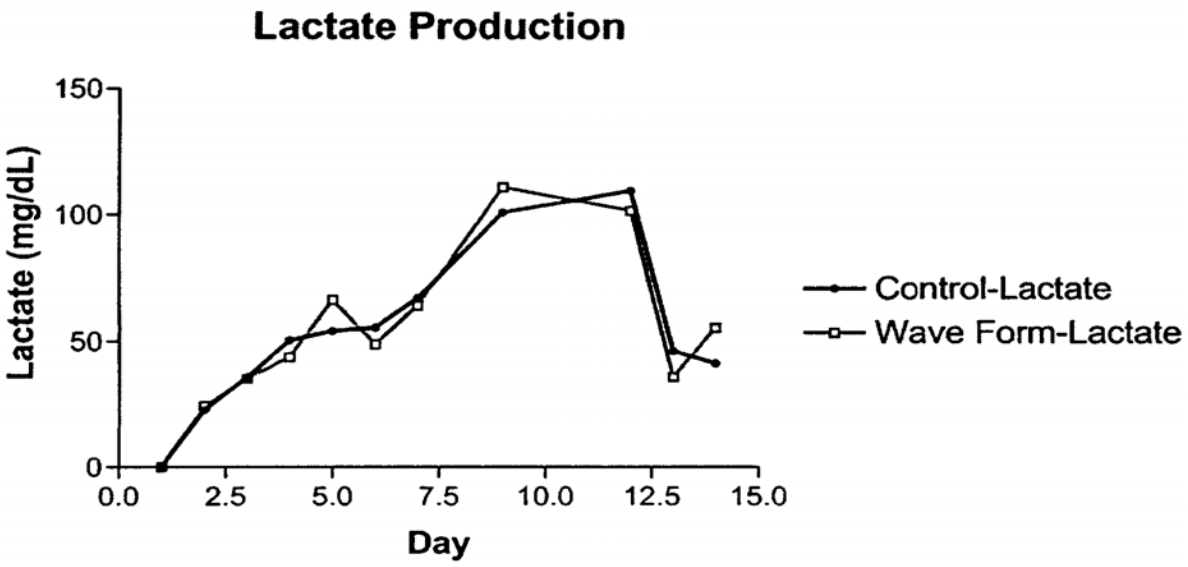

FIGURE 11. Lactate production in the cultures was nearly identical. Comparison with glucose consumption indicates the expected parallel.

Gene Array

NHNP cells were exposed to TVEMF and non-waveform growth conditions for 17-21 days. Upon completion of the exposure period, cells were harvested via trypsinization and poly-RNA was prepared from the respective groups of cells. RNA samples were quick frozen and subjected to the Gene Discovery Array from the I.M.A.G.E. Consortium (LLNL)1 cDNA analysis as described above. The tables below show the results of a survey of the response of over 10,000 human genes. The results are divided into two categories and shown in Tables 5 and 6. Table 5 shows those genes that were down-regulated or suppressed by the TVEMF and Table 6 shows those up-regulated or enhanced in activity by the TVEMF.

Analysis of the data indicates a significant down-regulation of about 175 maturation and regulatory genes and up-regulation of about 150 genes associated with growth and cellular proliferation. The down-regulated genes are normally associated with the differentiated or non-growth profile of normal cells. Table 5 lists the down-regulated genes in descending order. The up-regulated genes are in the main associated with growth and cellular proliferation. Table 6 lists the up-regulated genes in ascending order.

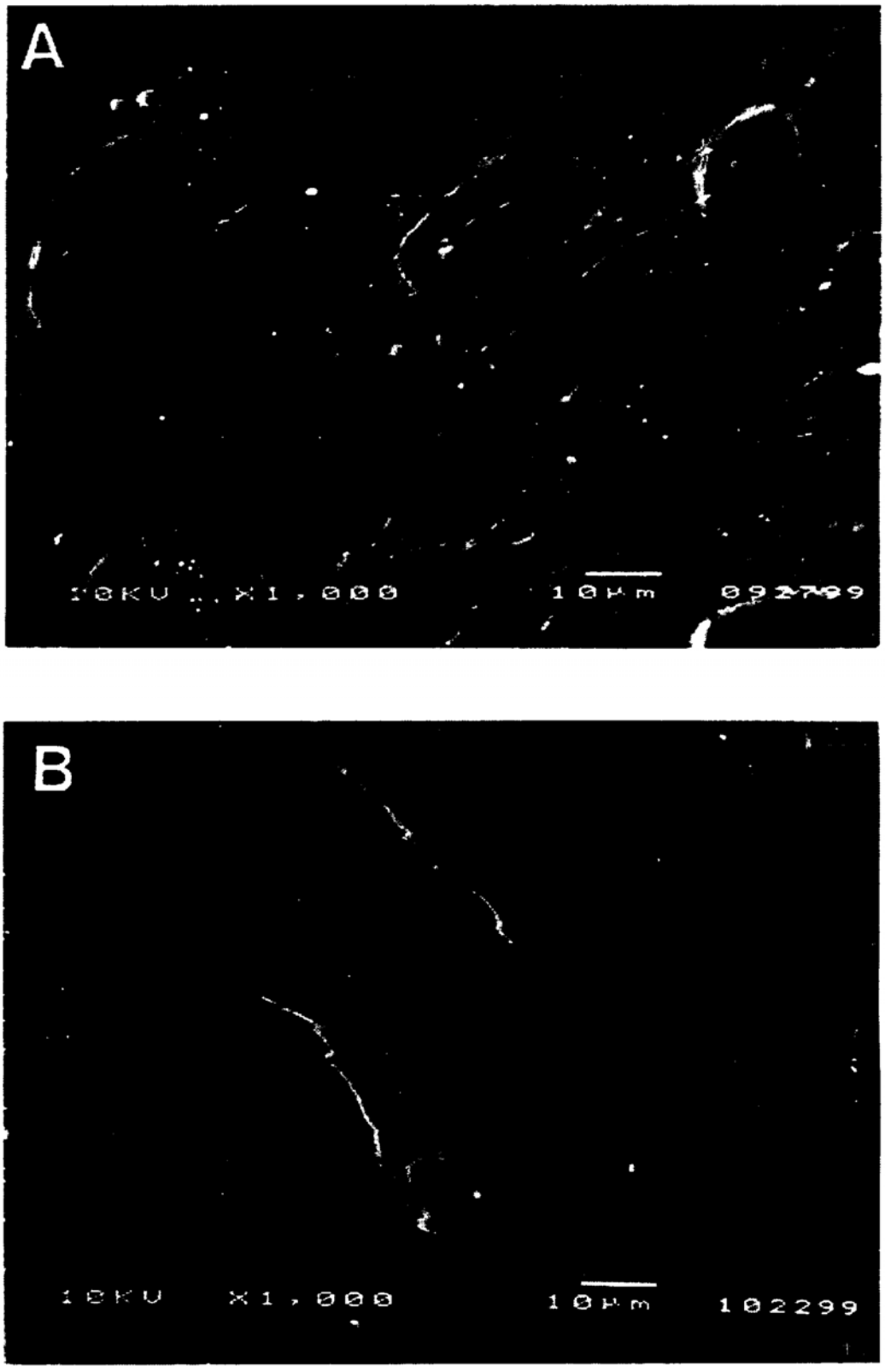

FIGURE 12. 12A shows NHNP cells grown without TVEMF; 12B shows the dramatic difference in the development of neural "tubes" under these conditions.

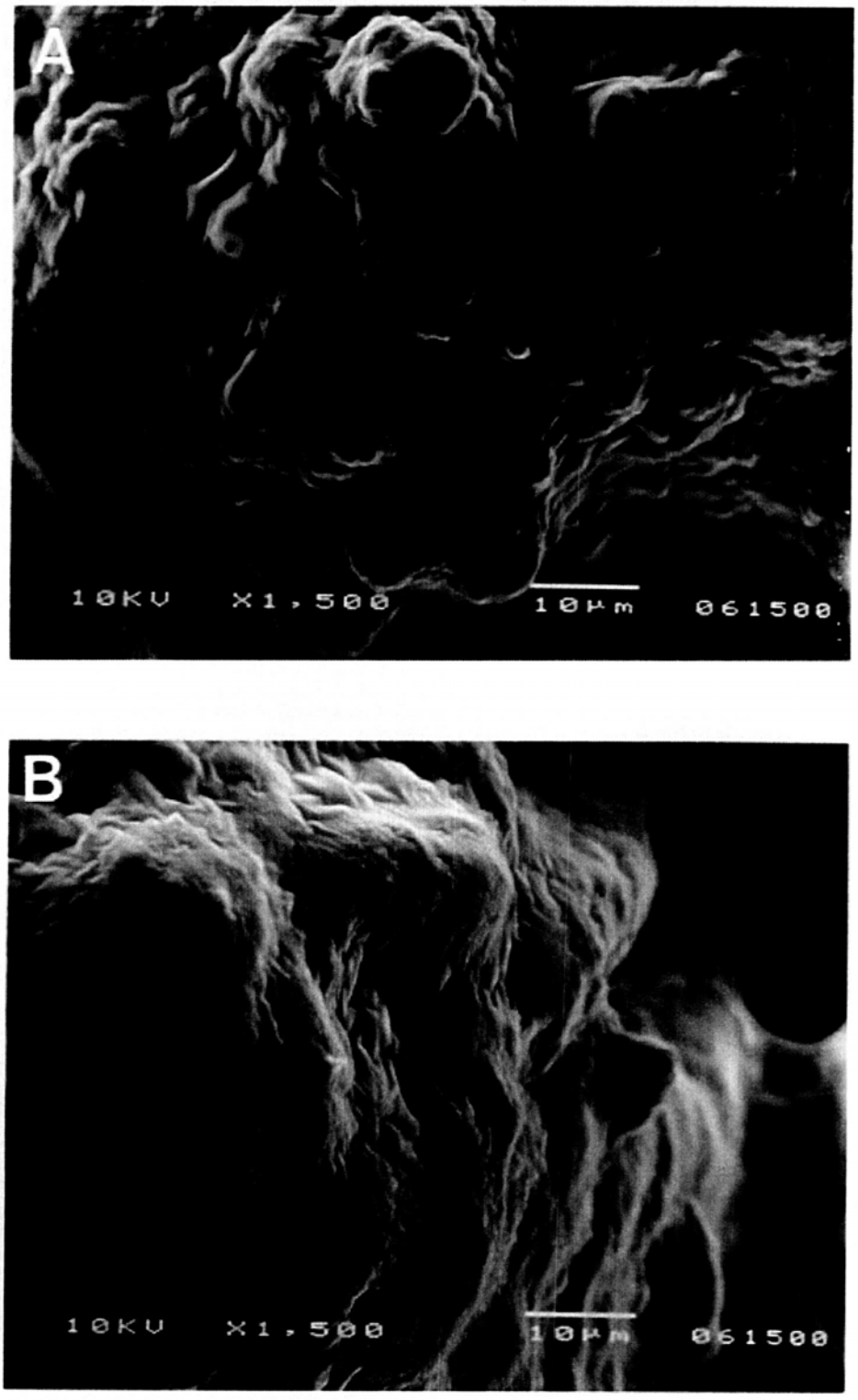

FIGURE 13. Observe the difference between 13A (untreated) and 13B. Cellular organization changed dramatically with TVEMF exposure in RWV three-dimensional culture.

Before this study, neither two- nor three-dimensional growth of neural cells had been achieved with the positive outcome of enhanced growth and apparent gene regulatory control. The genes in Table 5 were down-regulated by between -1.4 to -2.7 logs. The genes in Table 6 were up-regulated by between 1.7 to 8.4 logs. This demonstrates that the results presented are in no manner marginal.

TABLE 5. Down-Regulated Genes in Descending Order

Log form change	Gene
-2.7	Homo sapiens (clone Zap2) mRNA fragment {Incyte PD:1661837}
-2.5	CDC28 protein kinase 2 {Incyte PD:1384823}
-2.3	Synteni: YCFR 22 {YC 22.2000.W} /Unidentified
-2.3	ESTs, Moderately similar to cell growth regulating nucleolar protein LYAR [M.musculus] {Incyte PD:2233551}
-2.2	KERATIN, TYPE II CYTOSKELETAL 7 {Incyte PD:1649959}
-2.2	MITOTIC KINESIN-LIKE PROTEIN-1 {Incyte PD:2640427}
-2.1	EST {Incyte PD:674714}
-2.1	Synteni: YCFR 26 {YC 26.0062.N}} /Unidentified
-2	Synteni: YCFR 22 {YC 22.2000.X}} /Unidentified
-2	Synteni: YCFR 22 {YC 22.2000.Z}} /Unidentified
-1.9	Transcription factor 6-like 1 (mitochondrial transcription factor 1-like) {Incyte PD:3371995}
-1.9	Interferon-inducible 56-KDa protein {Incyte PD:1215596}
-1.9	EST {Incyte PD:1794375}
-1.9	Homo sapiens mitotic feedback control protein Madp2 homolog mRNA, complete cds {Incyte PD:2414624}
-1.9	EST {Incyte PD:151026}
-1.9	Homo sapiens Pig3 (PIG3) mRNA, complete cds {Incyte PD:2395269}
-1.9	Cellular retinoic acid-binding protein [human, skin, mRNA, 735 nt] {Incyte PD:585432}
-1.9	EST {Incyte PD:1755159}
-1.8	General transcription factor IIIA {Incyte PD:1527070}
-1.8	Carbonic anhydrase II {Incyte PD:2474163}
-1.8	EST {Incyte PD:660376}
-1.8	N-CHIMAERIN {Incyte PD:1852659}
-1.8	EST {Incyte PD:1798393}
-1.8	Chromosome condensation 1 {Incyte PD:3180854}
-1.7	Homo sapiens mRNA for KIAA0285 gene, complete cds {Incyte PD:1738053}
-1.7	ESTs, Weakly similar to F25H5.h [C.elegans] {Incyte PD:1923567}
-1.7	Homo sapiens mRNA expressed in osteoblast, complete cds {Incyte PD:2537863}
-1.7	EST {Incyte PD:3204745}
-1.7	Homo sapiens mRNA for serine/threonine protein kinase SAK {Incyte PD:2732630}
-1.7	Homo sapiens serum-inducible kinase mRNA, complete cds {Incyte PD:1255087}
-1.7	GRANCALCIN {Incyte PD:1671852}
-1.7	Homo sapiens Pig10 (PIG10) mRNA, complete cds {Incyte PD:1731061}
-1.7	Adenylosuccinate lyase {Incyte PD:1653326}
-1.7	Homo sapiens HP protein (HP) mRNA, complete cds {Incyte PD:3084122}
-1.7	ESTs, Moderately similar to T10C6.i [C.elegans] {Incyte PD:1923186}
-1.7	Calmodulin 1 (phosphorylase kinase, delta) {Incyte PD:2803306}

Log form change	Gene
-1.7	Centromere protein A (17kD) {Incyte PD:2444942}
-1.7	V-jun avian sarcoma virus 17 oncogene homolog {Incyte PD:1920177}
-1.7	Human glutathione-S-transferase homolog mRNA, complete cds {Incyte PD:1862232}
-1.7	Homo sapiens gene for protein involved in sexual development, complete cds {Incyte PD:3033934}
-1.7	EST {Incyte PD:2630992}
-1.7	Human low-Mr GTP-binding protein (RAB32) mRNA, partial cds {Incyte PD:1662688}
-1.7	Hydroxymethylbilane synthase {Incyte PD:1509204}
-1.7	Ribosomal protein L7a {Incyte PD:2579602}
-1.7	Human mRNA for myosin regulatory light chain {Incyte PD:78783}
-1.7	Ferredoxin reductase {Incyte PD:1819763}
-1.7	Human copper transport protein HAH1 (HAH1) mRNA, complete cds {Incyte PD:2313349}
-1.7	Synteni: HK 4 {HK 4.2000.W} } /Unidentified
-1.7	Human XIST, coding sequence a mRNA (locus DXS399E) {Incyte PD:1514318}
-1.7	Human mRNA for Apo1_Human (MER5(Aop1-Mouse)-like protein), complete cds {Incyte PD:2527879}
-1.7	Synteni: HK 4 {HK 4.2000.Z} } /Unidentified
-1.7	Human PINCH protein mRNA, complete cds {Incyte PD:126888}
-1.7	Homo sapiens peroxisome assembly protein PEX10 mRNA, complete cds {Incyte PD:998279}
-1.7	Homo sapiens short chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) mRNA, complete cds {Incyte PD:1638850}
-1.7	Neuroblastoma RAS viral (v-ras) oncogene homolog {Incyte PD:2816984}
-1.7	Human forkhead protein FREAC-1 mRNA, complete cds {Incyte PD:1449920}
-1.6	Annexin III (lipocortin III) {Incyte PD:1920650}
-1.6	Synteni: HK 4 {HK 4.2000.Y} } /Unidentified
-1.6	Human G protein gamma-11 subunit mRNA, complete cds {Incyte PD:1988432}
-1.6	Ribosomal protein, large, P0 {Incyte PD:3511355}
-1.6	Homo sapiens clone 23714 mRNA sequence {Incyte PD:1728368}
-1.6	Proteasome (prosome, macropain) subunit, beta type, 5 {Incyte PD:2503119}
-1.6	H.sapiens mRNA for b4 integrin interactor {Incyte PD:1932850}
-1.6	Human mRNA for protein D123, complete cds {Incyte PD:1920522}
-1.6	Homo sapiens Na+/Ca+ exchanger mRNA sequence {Incyte PD:2880435}
-1.6	STRESS-ACTIVATED PROTEIN KINASE JNK1 {Incyte PD:3331719}
-1.6	Homo sapiens leupaxin mRNA, complete cds {Incyte PD:1595756}
-1.6	EST {Incyte PD:1798965}
-1.6	Human DNA from overlapping chromosome 19 cosmids R31396, F25451, and R31076 containing COX6B and UPKA, genomic sequence {Incyte PD:1320685}
-1.6	INTERFERON-INDUCED 17 KD PROTEIN {Incyte PD:2862971}
-1.6	Human homolog of yeast IPP isomerase {Incyte PD:1526240}
-1.6	Translation elongation factor 1 gamma {Incyte PD:3138196}
-1.6	Tropomyosin alpha chain (skeletal muscle) {Incyte PD:1572555}
-1.6	Aplysia ras-related homolog 9 {Incyte PD:2733928}
-1.6	Homo sapiens androgen receptor associated protein 24 (ARA24) mRNA, complete cds {Incyte PD:552654}
-1.6	Human enhancer of rudimentary homolog mRNA, complete cds {Incyte PD:1704472}
-1.6	Ubiquitin-like protein {Incyte PD:1754454}
-1.6	Ornithine decarboxylase 1 {Incyte PD:1930235}

Log form change	Gene
-1.6	EST {Incyte PD:3605632}
-1.6	EST {Incyte PD:2057260}
-1.6	ESTs, Weakly similar to CAMP-DEPENDENT PROTEIN KINASE TYPE 2 [Saccharomyces cerevisiae] {Incyte PD:2055611}
-1.6	ESTs, Weakly similar to K01H12.1 [C.elegans] {Incyte PD:56197}
-1.6	Cell division cycle 2, G1 to S and G2 to M {Incyte PD:1525795}
-1.6	EST {Incyte PD:1794175}
-1.6	EST {Incyte PD:1489557}
-1.6	ESTs, Weakly similar to transcription factor [H.sapiens] {Incyte PD:1637517}
-1.6	RAS-LIKE PROTEIN TC21 {Incyte PD:2505425}
-1.6	Proliferating cell nuclear antigen {Incyte PD:2781405}
-1.6	Human mRNA for proteasome subunit HsC10-II, complete cds {Incyte PD:1737833}
-1.6	Human stimulator of TAR RNA binding (SRB) mRNA, complete cds {Incyte PD:2057162}
-1.6	EST {Incyte PD:2507206}
-1.6	H.sapiens mRNA for Ndr protein kinase {Incyte PD:3318571}
-1.6	ESTs, Weakly similar to Grb2-related adaptor protein [H.sapiens] {Incyte PD:1857259}
-1.6	ESTs, Highly similar to Tbc1 [M.musculus] {Incyte PD:1889147}
-1.6	GTPase-activating protein ras p21 (RASA) {Incyte PD:147344}
-1.6	Synteni: YCFR 22 {YC 22.2000.Y} } /Unidentified
-1.6	Human non-histone chromosomal protein (NHC) mRNA, complete cds {Incyte PD:1748670}
-1.6	Thioredoxin {Incyte PD:2606240}
-1.6	Homo sapiens heat shock protein hsp40 homolog mRNA, complete cds {Incyte PD:2844989}
-1.6	Homo sapiens DNA binding protein homolog (DRIL1) mRNA, complete cds {Incyte PD:2538333}
-1.5	H.sapiens mRNA for A-kinase anchoring protein AKAP95 {Incyte PD:1628787}
-1.5	Carbonyl reductase {Incyte PD:1633249}
-1.5	EST {Incyte PD:2060973}
-1.5	ESTs, Highly similar to GUANINE NUCLEOTIDE-BINDING PROTEIN G(I)/G(S)/G(O) GAMMA-7 SUBUNIT [Rattus norvegicus] {Incyte PD:1640161}
-1.5	CLEAVAGE SIGNAL-1 PROTEIN {Incyte PD:2054053}
-1.5	ATP SYNTHASE ALPHA CHAIN, MITOCHONDRIAL PRECURSOR {Incyte PD:3206210}
-1.5	Glucagon {Incyte PD:1333075}
-1.5	TRANSCRIPTIONAL ENHANCER FACTOR TEF-1 {Incyte PD:2957175}
-1.5	Human RGP4 mRNA, complete cds {Incyte PD:617517}
-1.5	Cellular retinol-binding protein {Incyte PD:1612969}
-1.5	Human p37NB mRNA, complete cds {Incyte PD:1407110}
-1.5	Human mRNA for suppressor for yeast mutant, complete cds {Incyte PD:2888814}
-1.5	EST {Incyte PD:3142705}
-1.5	ESTs, Weakly similar to PROTEIN PHOSPHATASE PP2A, 72 KD REGULATORY SUBUNIT [H.sapiens] {Incyte PD:2379045}
-1.5	CAMP-DEPENDENT PROTEIN KINASE TYPE II-ALPHA REGULATORY CHAIN {Incyte PD:1649731}
-1.5	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein) {Incyte PD:2193246}
-1.5	Small nuclear ribonucleoprotein polypeptides B and B1 {Incyte PD:2071473}
-1.5	EST {Incyte PD:1922084}
-1.5	ESTs, Highly similar to HIGH MOBILITY GROUP-LIKE NUCLEAR PROTEIN 2

Log form change	Gene
	[Saccharomyces cerevisiae] {Incyte PD:2669174}
-1.5	EST {Incyte PD:1844150}
-1.5	Homo sapiens mRNA for ST1C2, complete cds {Incyte PD:3993007}
-1.5	Human dual specificity phosphatase tyrosine/serine mRNA, complete cds {Incyte PD:1514573}
-1.5	Human mRNA for KIAA0123 gene, partial cds {Incyte PD:1752436}
-1.5	FATTY ACID-BINDING PROTEIN, EPIDERMAL {Incyte PD:2537805}
-1.5	Proteasome component C2 {Incyte PD:2195309}
-1.5	Human amyloid precursor protein-binding protein 1 mRNA, complete cds {Incyte PD:1663083}
-1.5	Human Has2 mRNA, complete cds {Incyte PD:3602403}
-1.5	EST {Incyte PD:1749678}
-1.5	Homo sapiens golgi SNARE (GS27) mRNA, complete cds {Incyte PD:3279439}
-1.5	Synteni: YCFR 22 {YC 22.2000.N} } /Unidentified
-1.5	Acid phosphatase 1, soluble {Incyte PD:620871}
-1.5	Human clone 23840 mRNA, partial cds {Incyte PD:1830083}
-1.5	H.sapiens mRNA for protein-tyrosine-phosphatase (tissue type: foreskin) {Incyte PD:444957}
-1.5	Human B-cell receptor associated protein (hBAP) mRNA, partial cds {Incyte PD:2545562}
-1.5	ESTs, Highly similar to ring finger protein [H.sapiens] {Incyte PD:2860918}
-1.5	H.sapiens mRNA for CLPP {Incyte PD:2675481}
-1.5	APOPTOSIS REGULATOR BCL-X {Incyte PD:1855683}
-1.5	Sorting nexin 1 {Incyte PD:1508407}
-1.5	Human voltage dependent anion channel form 3 mRNA, complete cds {Incyte PD:2051154}
-1.5	Human DEAD-box protein p72 (P72) mRNA, complete cds {Incyte PD:1750553}
-1.5	Ras homolog gene family, member G (rho G) {Incyte PD:1342744}
-1.5	Human FEZ2 mRNA, partial cds {Incyte PD:2623268}
-1.5	Human homolog of Drosophila discs large protein, isoform 2 (hdlg-2) mRNA, complete cds {Incyte PD:2203554}
-1.5	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria) {Incyte PD:1695917}
-1.5	ENOYL-COA HYDRATASE, MITOCHONDRIAL PRECURSOR {Incyte PD:2235870}
-1.5	INTERFERON GAMMA UP-REGULATED I-5111 PROTEIN PRECURSOR {Incyte PD:2211625}
-1.5	H.sapiens RY-1 mRNA for putative nucleic acid binding protein {Incyte PD:1805712}
-1.5	Cytochrome c oxidase subunit VIIb {Incyte PD:2060789}
-1.5	EST {Incyte PD:661516}
-1.5	EST {Incyte PD:1251588}
-1.5	EST {Incyte PD:1665871}
-1.5	Homo sapiens arsenite translocating ATPase (ASNA1) mRNA, complete cds {Incyte PD:1666094}
-1.5	Human SnRNP core protein Sm D3 mRNA, complete cds {Incyte PD:1624865}
-1.5	Homo sapiens clone 23777 putative transmembrane GTPase mRNA, partial cds {Incyte PD:2554541}
-1.5	Human Ki nuclear autoantigen mRNA, complete cds {Incyte PD:1308112}
-1.5	PLACENTAL CALCIUM-BINDING PROTEIN {Incyte PD:1222317}
-1.5	PRE-MRNA SPLICING FACTOR SF2, P32 SUBUNIT PRECURSOR {Incyte PD:1552335}

Log form change	Gene
-1.5	Human clone C4E 1.63 (CAC)n/(GTG)n repeat-containing mRNA {Incyte PD:1928789}
-1.5	Human glioma pathogenesis-related protein (GliPR) mRNA, complete cds {Incyte PD:477045}
-1.5	Homeo box A9 {Incyte PD:459651}
-1.4	ESTs, Weakly similar to UBIQUITIN-ACTIVATING ENZYME E1 HOMOLOG [H.sapiens] {Incyte PD:1710472}
-1.4	Voltage-dependent anion channel 2 {Incyte PD:2189062}
-1.4	Human rap2 mRNA for ras-related protein {Incyte PD:3334979}
-1.4	Human mRNA for KIAA0008 gene, complete cds {Incyte PD:1970111}
-1.4	PROTEASOME COMPONENT C13 PRECURSOR {Incyte PD:2668334}
-1.4	H.sapiens mRNA for translin {Incyte PD:986855}
-1.4	EST {Incyte PD:1377794}
-1.4	ALCOHOL DEHYDROGENASE {Incyte PD:1634342}
-1.4	Proteasome (prosome, macropain) subunit, beta type, 6 {Incyte PD:2989852}
-1.4	Epimorphin {Incyte PD:3438987}
-1.4	EST {Incyte PD:1905120}
-1.4	33 KD HOUSEKEEPING PROTEIN {Incyte PD:1819287}
-1.4	Homo sapiens nuclear VCP-like protein NVLp.2 (NVL.2) mRNA, complete cds {Incyte PD:1445507}
-1.4	Homo sapiens inositol polyphosphate 4-phosphatase type II-alpha mRNA, complete cds {Incyte PD:3032739}
-1.4	Homo sapiens regulator of G protein signaling RGS12 (RGS) mRNA, complete cds {Incyte PD:3618382}
-1.4	Homo sapiens peroxisomal phytanoyl-CoA alpha-hydroxylase (PAHX) mRNA, complete cds {Incyte PD:4073867}

TABLE 6. Waveform Up-Regulated Genes in Ascending Order

Log Form change	Gene
1.7	NEUROMEDIN B PRECURSOR {Incyte PD:2754315}
1.7	Synteni: YCFR 21 {YC 21.0031.N} } /Unidentified
1.7	ATRIAL NATRIURETIC PEPTIDE CLEARANCE RECEPTOR PRECURSOR {Incyte PD:1353606}
1.7	EST {Incyte PD:565872}
1.7	Synteni: YCFR 46 Cy3 {YC 46.2000.Z} } /Unidentified
1.7	ESTs, Weakly similar to metaxin [H.sapiens] {Incyte PD:1754461}
1.7	Plasminogen {Incyte PD:2515873}
1.7	Human mRNA for CC chemokine LARC precursor, complete cds {Incyte PD:2220923}
1.7	Synteni: YCFR 21 {YC 21.0062.N} } /Unidentified
1.7	Homo sapiens Amplified in Breast Cancer (AIB1) mRNA, complete cds {Incyte PD:2634478}
1.7	Homo sapiens clone 24640 mRNA sequence {Incyte PD:1560143}
1.7	Synteni: YCFR 21 {YC 21.2000.N} } /Unidentified
1.7	EST {Incyte PD:143912}
1.7	Human transcription factor SIM2 long form mRNA, complete cds {Incyte PD:996104}
1.7	EST {Incyte PD:2841478}
1.7	PUTATIVE DNA BINDING PROTEIN A20 {Incyte PD:1878791}
1.7	Protein tyrosine phosphatase, receptor type, mu polypeptide {Incyte PD:987736}
1.7	Human clone A9A2BRB5 (CAC)n/(GTG)n repeat-containing mRNA {Incyte PD:1987975}
1.7	Endothelin converting enzyme 1 {Incyte PD:1963819}
1.7	Argininosuccinate synthetase {Incyte PD:1981145}
1.7	Human clone 46690 brain expressed mRNA from chromosome X {Incyte PD:1669780}
1.7	Human plectin (PLEC1) mRNA, complete cds {Incyte PD:1907232}
1.7	Homo sapiens mRNA for calmegin, complete cds {Incyte PD:2498216}
1.7	EST {Incyte PD:769182}
1.7	GLUCOSE TRANSPORTER TYPE 3, BRAIN {Incyte PD:2745082}
1.7	Human c-jun proto oncogene (JUN), complete cds, clone hCJ-1 {Incyte PD:1969563}
1.7	Microtubule-associated protein 1A {Incyte PD:702684}
1.7	Clusterin (complement lysis inhibitor; testosterone-repressed prostate message 2; apolipoprotein J) {Incyte PD:2966620}
1.7	NADH-CYTOCHROME B5 REDUCTASE {Incyte PD:1901142}
1.7	Protein-tyrosine kinase 7 {Incyte PD:996229}
1.7	Alpha-1 type XVI collagen {Incyte PD:1963529}
1.7	EST {Incyte PD:2839121}
1.7	Homo sapiens mRNA for DEC1, complete cds {Incyte PD:1732479}
1.8	Synteni: YCFR 85 {YC 85.2000.Y} } /Unidentified
1.8	Homo sapiens CHD3 mRNA, complete cds {Incyte PD:1965248}
1.8	BB1 {Incyte PD:1966148}
1.8	Pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1) {Incyte PD:2989411}
1.8	Human breast epithelial antigen BA46 mRNA, complete cds {Incyte PD:1319020}
1.8	Amyloid beta (A4) precursor-like protein 2 {Incyte PD:3876715}
1.8	Polymerase (RNA) II (DNA directed) polypeptide A (220kD) {Incyte PD:1382059}
1.8	Homo sapiens sarco-/endoplasmic reticulum Ca-ATPase 3 (ATP2A3) mRNA, alternatively spliced, partial cds {Incyte PD:688411}
1.8	Human endogenous retroviral protease mRNA, complete cds {Incyte PD:1347636}

Log Form change	Gene
1.8	ATPase, Na+/K+ transporting, alpha 1 polypeptide {Incyte PD:1730609}
1.8	Laminin, alpha 4 {Incyte PD:1851696}
1.8	Hexabrachion (tenascin C, cytotactin) {Incyte PD:1453450}
1.8	Human mRNA for KIAA0325 gene, partial cds {Incyte PD:1995315}
1.8	Integrin beta-5 subunit {Incyte PD:418731}
1.8	Microfibrillar-associated protein 4 {Incyte PD:1659231}
1.8	Fibulin 1 {Incyte PD:1320658}
1.8	Protein serine/threonine kinase stk2 {Incyte PD:1518981}
1.8	Human putative RNA binding protein (RBP56) mRNA, complete cds {Incyte PD:1907369}
1.8	Homo sapiens CAGH3 mRNA, complete cds {Incyte PD:1432042}
1.8	EST {Incyte PD:2953888}
1.8	Human mRNA for KIAA0062 gene, partial cds {Incyte PD:3138128}
1.8	Human clone HSH1 HMG CoA synthase mRNA, partial cds {Incyte PD:1807407}
1.8	Filamin 1 (actin-binding protein-280) {Incyte PD:1708528}
1.8	Synteni: YCFR 85 {YC 85.2000.X} } /Unidentified
1.8	Synteni: YCFR 46 Cy3 {YC 46.2000.W} } /Unidentified
1.8	Homologue of mouse tumor rejection antigen gp96 {Incyte PD:2679349}
1.8	Human XMP mRNA, complete cds {Incyte PD:1887661}
1.8	Cytochrome P450, subfamily XIA (cholesterol side chain cleavage) {Incyte PD:2368282}
1.8	78 KD GLUCOSE REGULATED PROTEIN PRECURSOR {Incyte PD:2884613}
1.9	ESTs, Weakly similar to HYPOTHETICAL 16.1 KD PROTEIN IN SEC17-QCR1 INTERGENIC REGION [Saccharomyces cerevisiae] {Incyte PD:1923722}
1.9	Homo sapiens carbonic anhydrase precursor (CA 12) mRNA, complete cds {Incyte PD:3766382}
1.9	H.sapiens mRNA for SIX1 protein {Incyte PD:3208486}
1.9	Plasminogen activator inhibitor, type I {Incyte PD:1445767}
1.9	Human mRNA for SHPS-1, complete cds {Incyte PD:2180684}
1.9	Collagen, type V, alpha 1 {Incyte PD:1672442}
1.9	Homo sapiens monocarboxylate transporter (MCT3) mRNA, complete cds {Incyte PD:1343253}
1.9	Human follistatin gene {Incyte PD:1577614}
1.9	Homo sapiens mRNA for PRP8 protein, complete cds {Incyte PD:3616229}
1.9	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor {Incyte PD:1556061}
1.9	Human p120E4F transcription factor mRNA, complete cds {Incyte PD:1940164}
1.9	Collagen, type VI, alpha 1 {Incyte PD:2672056}
1.9	Human mRNA for pM5 protein {Incyte PD:1578951}
1.9	ALZHEIMER'S DISEASE AMYLOID A4 PROTEIN PRECURSOR {Incyte PD:126370}
1.9	Tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory) {Incyte PD:418041}
1.9	Granulin {Incyte PD:812141}
1.9	Human extracellular matrix protein 1 (ECM1) mRNA, complete cds {Incyte PD:1965806}
1.9	Synteni: YCFR 21 {YC 21.2000.X} } /Unidentified
1.9	Homo sapiens mRNA for serin protease with IGF-binding motif, complete cds {Incyte PD:1958902}
1.9	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein {Incyte PD:1687060}
1.9	Fibulin 2 {Incyte PD:1901095}
1.9	Kinase insert domain receptor (a type III receptor tyrosine kinase) {Incyte PD:2220338}
1.9	Synteni: YCFR 45 {YC 45.2000.X} } /Unidentified
1.9	Cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase) {Incyte PD:168865}

Log Form change	Gene
1.9	Latent transforming growth factor beta binding protein 1 {Incyte PD:1313183}
1.9	Lysyl hydroxylase {Incyte PD:1759127}
1.9	Human mRNA for KIAA0230 gene, partial cds {Incyte PD:1449824}
1.9	H.sapiens garp gene mRNA, complete CDS {Incyte PD:3572014}
1.9	EST {Incyte PD:724880}
1.9	ESTs, Weakly similar to TRANSMEMBRANE PROTEIN SEX PRECURSOR [H.sapiens] {Incyte PD:1511346}
2	Solute carrier family 6 (neurotransmitter transporter, taurine), member 6 {Incyte PD:1516886}
2	Hormone receptor (growth factor-inducible nuclear protein N10) {Incyte PD:1958560}
2	Syndecan 4 (amphiglycan, ryudocan) {Incyte PD:3214670}
2	Synteni: YCFR 21 {YC 21.0500.N} } /Unidentified
2	Human pre-B cell enhancing factor (PBEF) mRNA, complete cds {Incyte PD:1641590}
2	Human mRNA for dihydropyrimidinase related protein-2, complete cds {Incyte PD:2784546}
2	EST {Incyte PD:2580841}
2	Protein kinase C substrate 80K-H {Incyte PD:1723971}
2.1	Human contactin associated protein (Caspr) mRNA, complete cds {Incyte PD:2309047}
2.1	Human cysteine-rich fibroblast growth factor receptor (CFR-1) mRNA, complete cds {Incyte PD:2204871}
2.1	Collagen, type V, alpha {Incyte PD:1887959}
2.1	H.sapiens RNA for type VI collagen alpha3 chain {Incyte PD:1314882}
2.2	Fibrillin 1 (Marfan syndrome) {Incyte PD:1448051}
2.2	Collagen, type XI, alpha 1 {Incyte PD:3598222}
2.2	H.sapiens mRNA for extracellular matrix protein collagen type XIV, C-terminus {Incyte PD:2208990}
2.2	Collagen, type II, alpha 1 (primary osteoarthritis, spondyloepiphyseal dysplasia, congenital) {Incyte PD:2518178}
2.2	ESTs, Weakly similar to unknown [S.cerevisiae] {Incyte PD:2171401}
2.2	EST {Incyte PD:1923572}
2.3	Human insulin-like growth factor binding protein 5 (IGFBP5) mRNA {Incyte PD:1686585}
2.3	Human mRNA for KIAA0242 gene, partial cds {Incyte PD:1940994}
2.3	Complement component 1, s subcomponent {Incyte PD:1904751}
2.3	Human chromosome 17 unknown product mRNA, complete cds {Incyte PD:2849603}
2.3	Homo sapiens lysosomal pepstatin insensitive protease (CLN2) mRNA, complete cds {Incyte PD:3500996}
2.3	Collagen, type IV, alpha 2 {Incyte PD:1906574}
2.3	ESTs, Moderately similar to ZINC FINGER PROTEIN HF.12 [Homo sapiens] {Incyte PD:3729155}
2.3	P55-C-FOS PROTO-ONCOGENE PROTEIN {Incyte PD:341491}
2.4	Homo sapiens stanniocalcin precursor (STC) mRNA, complete cds {Incyte PD:2222921}
2.4	EST {Incyte PD:2424631}
2.5	EST {Incyte PD:1940710}
2.6	Thrombospondin 1 {Incyte PD:2055534}
2.6	INTEGRAL MEMBRANE PROTEIN E16 {Incyte PD:1911012}
2.6	Collagen, type I, alpha 1 {Incyte PD:782235}
2.7	Complement component C1r {Incyte PD:1664320}
2.7	REGULATOR OF G-PROTEIN SIGNALLING 2 {Incyte PD:1218114}
2.7	H.sapiens mRNA for adipophilin {Incyte PD:1985104}
2.7	EST {Incyte PD:1979450}
2.7	EST {Incyte PD:690994}

Log Form change	Gene
2.8	Cathepsin D (lysosomal aspartyl protease) {Incyte PD:3940755}
2.8	Matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase) {Incyte PD:1558081}
3	Cyclin D2 {Incyte PD:1618422}
3.2	EST {Incyte PD:2636514}
3.2	COMPLEMENT C3 PRECURSOR {Incyte PD:1513989}
3.2	Fibronectin 1 {Incyte PD:3553729}
3.3	Homo sapiens secreted frizzled related protein mRNA, complete cds {Incyte PD:428236}
3.3	INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 PRECURSOR {Incyte PD:1447903}
3.3	Human autoantigen DFS70 mRNA, partial cds {Incyte PD:42920}
3.4	Early growth response protein 1 {Incyte PD:1705208}
3.4	Synteni: YCFR 43 {YC 43.2000.W}
3.5	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) {Incyte PD:3139163}
3.6	Synteni: YCFR 43 {YC 43.2000.Z} } /Unidentified
3.8	Synteni: YCFR 23 {YC 23.0062.N} } /Unidentified
4.1	Synteni: YCFR 43 {YC 43.2000.Y} } /Unidentified
4.2	Homo sapiens phosphomevalonate kinase mRNA, complete cds {Incyte PD:1497123}
4.3	Synteni: YCFR 43 {YC 43.2000.X} } /Unidentified
4.7	Synteni: YCFR 23 {YC 23.0031.N} } /Unidentified
5.4	CARTILAGE GLYCOPROTEIN-39 PRECURSOR {Incyte PD:157510}
6.7	Synteni: YCFR 23 {YC 23.0125.N} } /Unidentified
6.9	Synteni: YCFR 23 {YC 23.0250.N} } /Unidentified
7.3	Synteni: YCFR 23 {YC 23.4000.N} } /Unidentified
8.4	Human germline oligomeric matrix protein (COMP) mRNA, complete cds {Incyte PD:2636634}

DISCUSSION AND CONCLUSIONS

The ability to use TVEMF to control the proliferative rate, directional attitude, and molecular genetic expression of normal human neural progenitor cells has been demonstrated. The procedure is applicable to, but not limited to, the control of NHNP cells in both two-dimensional and three-dimensional culture. As presented in the molecular genetic data shown in Tables 5 and 6, many of the genetic responses in both up-regulated and down-regulated genes are maturation and growth regulatory in nature. These genes are also primarily involved in the embryogenic process. Therefore it is reasonable to conclude that control over the embryogenic development process may be achieved via the presently demonstrated methodology.

As shown in Table 6, specific genes such as human germline oligomeric matrix protein, prostaglandin endoperoxide synthase 2, early growth response protein 1, and insulin-like growth factor binding protein 3 precursor are highly up-regulated. Table 5 shows Keratin Type II cytoskelatal 7, mytotic kinesin like protein 1, transcription factor 6 like 1, mytotic feedback

control protein, and cellular retinoic acid binding protein are down-regulated. Each of these two sets is only an example from the approximately 320 genes changes expressed as a consequence of exposure to TVEMF.

There is significant precedent in the literature for the results reported above. Kepler et al. (1990) reported the effects of the neurons with oscillatory properties on the composite of neural networks. This work illustrates the likelihood that a pulse width modulated system might bring on specific responses in neural tissues. As previously discussed, Valentini et al. (1993) demonstrated the ability to enhance the outgrowth of neural fibers on materials that possess a weak electric charge. This would indicate that intense electric fields are not necessarily an essential component of this process, and that a weak and persistent stimulus might yield a measurable effect.

Additional evidence of the effects of magnetic fields exists in the work of Sandyk et al. (1992a). This communication details dramatic improvement of a patient with progressive degenerative multiple sclerosis. Briefly, the patient showed considerable improvement when subjected to treatment at a frequency of 2-7 Hz and an intensity of the magnetic field of 7.5 pico Tesla. These parameters marginally parallel those of this report. In a similar fashion, Sandyk et al. (1992b) reported significant improvement in patents treated with the same field strength and intensity.

The ability to suppress or stimulate the growth of non-excitable cells has been reported in mouse lymphoma cells by Lyte et al. (1991). A narrow range of electric field was found to be effective at one end to stimulate and at the other to inhibit the growth of these cells. These data might suggest cellular receptors in all cells. To sustain this notion, Brüstle et al. (1996) reported the potential to use neural progenitors for recapitulation of neural tissues. As would be expected, this would require genetic control at the embryonic level. We believe this study indicates our ability to trigger these parametric events.

As is clearly demonstrated in the human body, the bioelectric, biochemical process of electrical nerve stimulation is a documented reality. The present investigation demonstrates that a similar phenomenon can be potentiated in a synthetic atmosphere, i.e., two-dimensionally or in rotating wall cell culture vessels. One may use this electrical potentiation for a number of purposes, including developing tissues for transplantation, repairing traumatized tissues, and moderating some neurodegenerative diseases and perhaps controlling the degeneration of tissue as might be effected in a bioelectric stasis field.

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The present investigation details the development of model systems for growing two and three-dimensional human neural progenitor cells within a culture medium facilitated by a time-varying electromagnetic field (TVEMF). The cells and culture medium are contained within a two or three-dimensional culture vessel, and the electromagnetic field is emitted from an electrode or coil. These studies further provide methods to promote neural tissue regeneration by means of culturing the neural cells in either configuration. Grown in two-dimensions, neuronal cells extended longitudinally forming tissue strands extending axially along and within electrodes comprising electrically conductive channels or guides through which a time-varying electrical current was conducted. In the three-dimensional aspect exposure to TVEMF resulted in the development of three-dimensional aggregates, which emulated organized neural tissues. In both experimental configurations, the proliferation rate of the TVEMF cells was 2.5 to 4.0 times the rate of the non-waveform cells. Each of the experimental embodiments resulted in similar molecular genetic changes regarding the growth potential of the tissues as measured by gene chip analyses, which measured more than 10,000 human genes simultaneously

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