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Effect of Electromagnetic Stimulation With Different Waveforms on Cultured Chick Tendon Fibroblasts

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An energy efficient electromagnetic stimulator device for fracture healing was compared to a commercially available device in stimulating cell growth in tissue cultures. The energy efficient device, which conserves energy by using a bidirectional time-dependent magnetic wave form, and the commercially available stimulator, which uses a unidirectionaltime-dependent magnetic wave form, were tested on chick tendon fibroblasts in primary culture. Comparing non-stimulated control and cells electromagnetically stimulated with unidirectional and bidirectional waveforms showed that at the growth phase between days 2 and 3, both electrical stimulation techniques increased cell division as measured by DNA synthesis. When cells were dividing rapidly, collagen synthesis was reduced. When the cells reached the confluence there was no difference among the groups (control, unidirectionally stimulated, and bidirectionally stimulated) in terms of number of cells or collagen produced. ©1994 Wiley-Liss, Inc.

Key words: tissue culture, energy efficient device, DNA synthesis, frequency spectrum, collagen production, collagen synthesis

INTRODUCTION

It has been hypothesized that bio-feedback systems, which are based on bioelectric signals occurring in connective tissue growth and remodeling, are related to the electromechanical properties of the materials participating in the process [Bassett, 1971; Eriksson, 1976; Guzelsu, 1982].

The development of electromagnetic field parameters to treat skeletal and other disorders was based specifically on induced electric field waveform patterns [Bassett

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and Becker, 1962; Cochran et al., 1968] after the discovery of the electromechanical properties of bone tissue [Fukada and Yasuda, 1957; Eriksson, 1974]. Different techniques such as direct current [Brighton et al., 1975; Brighton et al., 1981], alternating current [Brighton and Pollack, 1984], and pulsed electromagnetic field (PEMF) [Bassett et al., 1981a,b; Watson and Downes, 1983; Downes and Watson, 1984; DeHaas et al., 1986; Borsalino et al., 1988] have been used to stimulate bone healing. Electrical and electromagnetic stimulation techniques have also been applied to the treatment of soft connective tissue disorders [Frank and Szeto, 1983; Binder et al., 1984; Akai et al., 1988; Ieran et al., 1990].

The non-invasive PEMF method generates an induced electric field in the tissue due to a time dependent magnetic induction (B field) at the placement site either by an air coil [Bassett et al., 1981a,b; Parkinson, 1985; DeHaas et al., 1986] or by an iron core electromagnet [Watson and Downes, 1983; Downes and Watson, 1984; Parkinson, 1985]. The early clinical units for bone healing were energy inefficient. This led to the development of devices that were powered from wall outlets [Bassett et al., 1981b], restricting the patients' mobility during the treatment. After the mid-1980s, portable battery-powered units became commercially available. Several methods were used to make these devices more energy efficient and portable, including the use of lower magnetic induction intensity, reduced distance between the poles of the magnet, and the use of an energy recovery system. In addition, most PEMF devices currently in clinical use have Fourier spectra in which low frequencies predominate.

Two techniques have been developed to increase the energy efficiency of the iron-core electromagnetic PEMF devices. The first one depends on an energy recovery system. In this technique, energy is recovered and stored during the decline of the B field [Thaler, 1987]. The second energy conservation technique depends upon the changes in the time-dependent magnetic induction wave form by using a magnetically bidirectional pulsed wave. This technique can reduce the energy consumption by approximately 50% as compared to commercially available devices having unidirectional wave forms. Although this second technique (bidirectional wave form) reduces the energy consumption, it affects the induced electric field wave shape. Therefore, one has to investigate the effectiveness of this wave shape (magnetic bidirectional) as compared to the traditional wave form (unidirectional) produced by commercially available devices. The first energy recovery system (recovery of energy during the decline of the field) described above can be incorporated into both systems (bidirectional and unidirectional) and in itself does not affect significantly the wave shape of the device.

In order to measure the effect of electrical stimulation on connective tissues, not only fibroblasts [Liboff et al., 1984; Murray and Farndale, 1985; Bourguignon and Bourguignon, 1987; McLeod et al., 1987; Murray et al., 1988] but also chondrocytes [Norton et al., 1977; Rodan et al., 1978; Norton, 1985; Brighton and Townsend, 1988; Elliott et al., 1988; Norton and Rovetti, 1988] and osteoblasts [Korenstein et al., 1984; Binderman et al., 1985; Stein et al., 1989] have been used in tissue culture experiments.

The goal of the present investigation is to examine the effect of a unidirectional PEMF device vs. a bidirectional energy efficient device on chick tendon cultured fibroblasts. In this study, DNA production, collagen production and synthesis, and cellular cAMP levels were measured.

MATERIALS AND METHODS

Design of Bidirectional Magnetic Field Generation

Bidirectional and unidirectional magnetic generators were built with an energy recovery design through which they recover energy during the decline of B field [Thaler, 1987]. The unidirectional wave shape device was designed to be similar to that employed by Cadossi and his co-workers [Cadossi et al., 1985; Borsalino et al., 1988; Ieran et al., 1990] and is commercially available (IGEA Corp.).

A block diagram of an energy recovery design for the bidirectional stimulator is shown in Figure 1. In Figure 1 to initiate the cycle, the logic generator closes SW_1, (the magnetic field starts; point 1, Fig. 3c) for a predetermined interval (up to point 2, Fig. 3c). The greater inductance of the n_1 winding provides a slower ramp (Slope S_1 = B/A) of magnetic field and a concomitantly smaller induced voltage (V_2 in Fig. 3d). After the appropriate time, SW_1 is opened (point 2, Fig. 3c) and a faster field collapse (slope S_2 = B/C) is accomplished by the lower inductance of the n_2 winding (from point 2 to 3, Fig. 3c). This provides a higher induced electric field (V_1 in Fig. 3d). Up to when the magnetic field crosses zero (point 3) the field energy has been recovered (between 2 and 3, Fig. 3c) and restored to the battery (through D2). SW_2 is then closed (point 3 through 4, Fig. 3c), causing the field to ramp negative at a faster rate (S_2) through n_2 winding prior to subsequent closing of D_1 to complete the cycle by slowly discharging the field through the high inductance (n_1) (points 4 through 5, Fig. 3c) to restore the bulk of the field energy to the battery (SW_2 is opened at point 4, Fig. 3c). The cycle then repeats itself.

In both systems magnetic semi-circular generators were built with a 27.7 cm internal diameter and 32.8 cm outer diameter (Fig. 2 B_1-A_1 diameter in side view). The coils were 11.0 cm wide (Fig. 2 front view width). These dimensions allowed us to place three 3.5 cm tissue culture plates in a plane (parallel to 1-1, 2-2, etc.

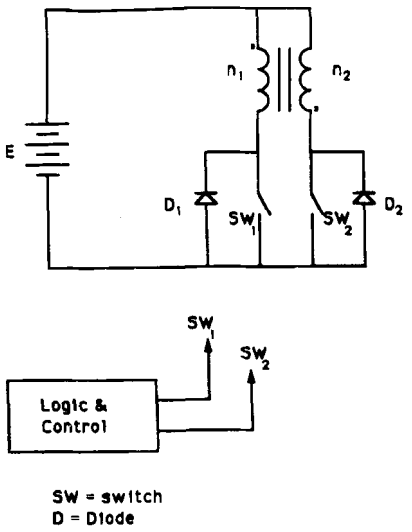

Fig. 1. Schematic diagram of bidirectional magnetic field generator which utilizes recovery of the field energy (energy recovery system) during the declining segments of the magnetic field (between points 2 and 3, 4 and 5 in Fig. 3c).

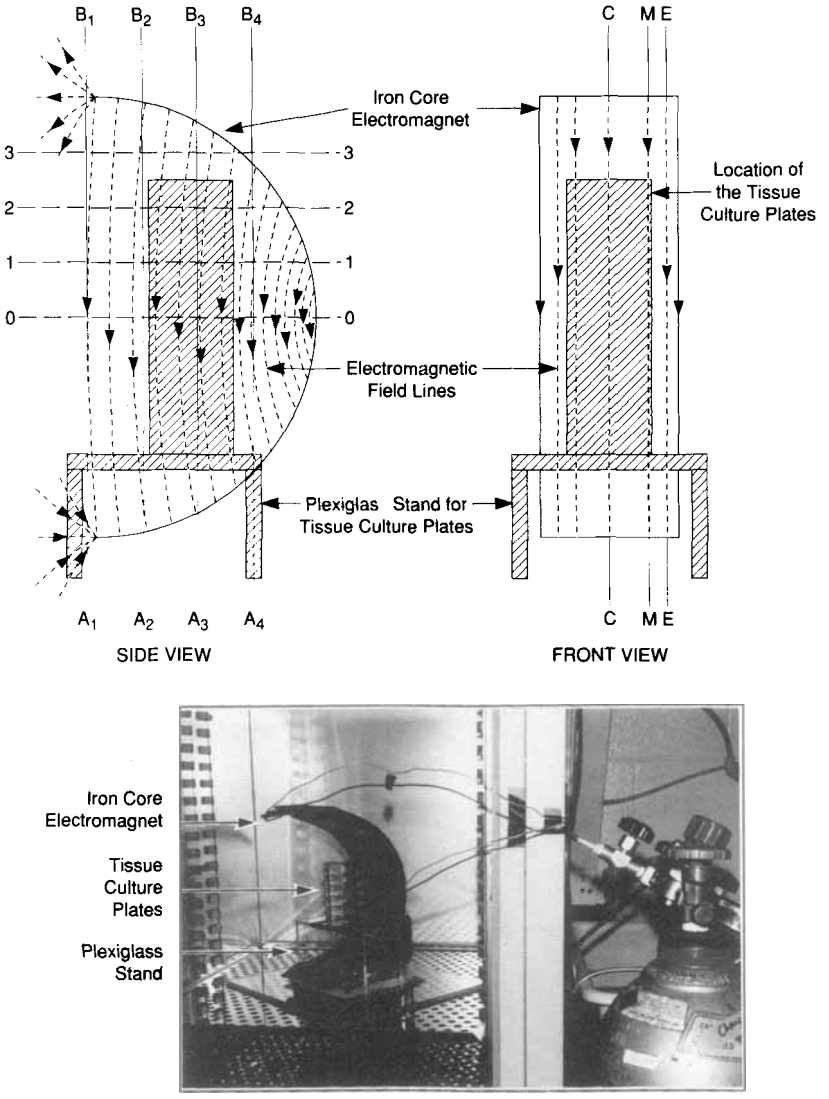

Fig. 2. **Upper:** The schematic for an electromagnetic coil in an incubator. The darker area in the middle indicates where the tissue culture plates were located. The tissue culture plates stack up on a Plexiglas stand which was located inside the electromagnet. Measurements were taken at all these points with a search coil for finding the average value of the magnetic field. **Lower:** Photography shows the overall experimental setup in the incubator.

planes in Fig. 2, side view) in the coils. The location of the stack of tissue culture plates is shown with the marked area in Figure 2 (front and side views).

The wave shapes of the unidirectional and bidirectional devices are shown in Figure 3. In Figure 3 the absolute maximum values of the magnetic field intensity (Ba) are almost the same for both systems. The slope ratio is S_1/S_2 (S_1 = B/A, S_2 = B/C, S_1/S_2 = C/A) and the duty cycle is defined as M/T (T Period). Repetition rate is defined as 1/T. Physical parameters were chosen for both systems S_1/S_2 = 3.0, M/T = 0.453, and 1/T = 76.9 Hz.

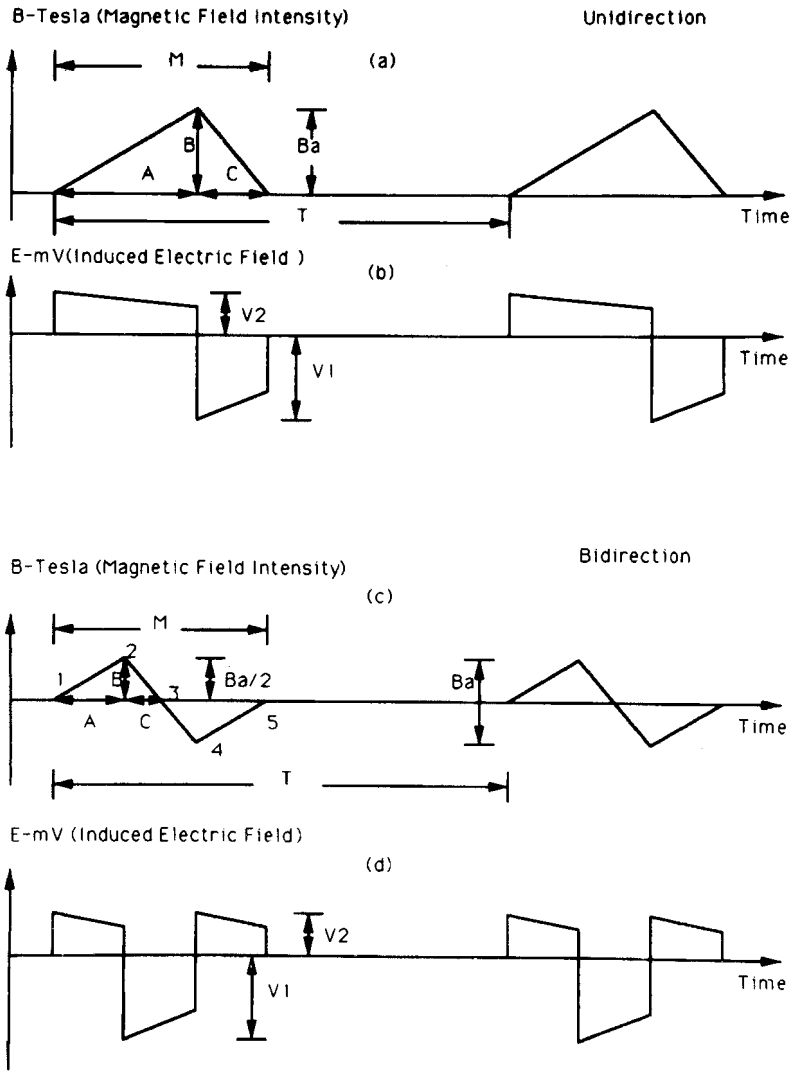

Fig. 3. The magnetic field intensity generated by the coils and induced electric field in the tissue culture plates. **a:** Magnetic field intensity for unidirectional system (20.33×10^{-4} Tesla average Ba). **b:** Induced electric field for unidirectional system (28.6×10^{-3} mV/cm average V_1). **c:** Magnetic field intensity for bidirectional system (21.54×10^{-4} Tesla average Ba). **d:** Induced electric field for bidirectional system (30.2×10^{-3} mV/cm average V_1).

The PEMF devices in general try to minimize the dissipative losses and maximize the resulting magnetic field. In our systems, unidirectional design yielded approximately 70% and bidirectional system yielded approximately 80% recoverable (non-dissipative) energy. The non-dissipative energy consumption of a PEMF device is related to the square of the magnetic induction amplitude (B_a^2). Therefore, for the same frequency and duty cycle, the energy efficient design (bidirectional) requires half the energy consumption ($B_a^2/4 + B_a^2/4 = B_a^2/2$) as the unidirectional system if they have exact symmetrical wave shapes (Fig. 3a,c). The

induced electric field for both systems appears different in time (Fig. 3b,d). In further theoretical analysis using Fourier transforms on the wave shapes of Figure 3a,c, it is apparent that the frequency components of the two systems do not differ from each other significantly (Fig. 4). The unidirectional signal has somewhat more energy in the low frequency range (below 76.9 Hz) compared to the bidirectional wave shape where its energy is confined more above the 76.9 Hz.

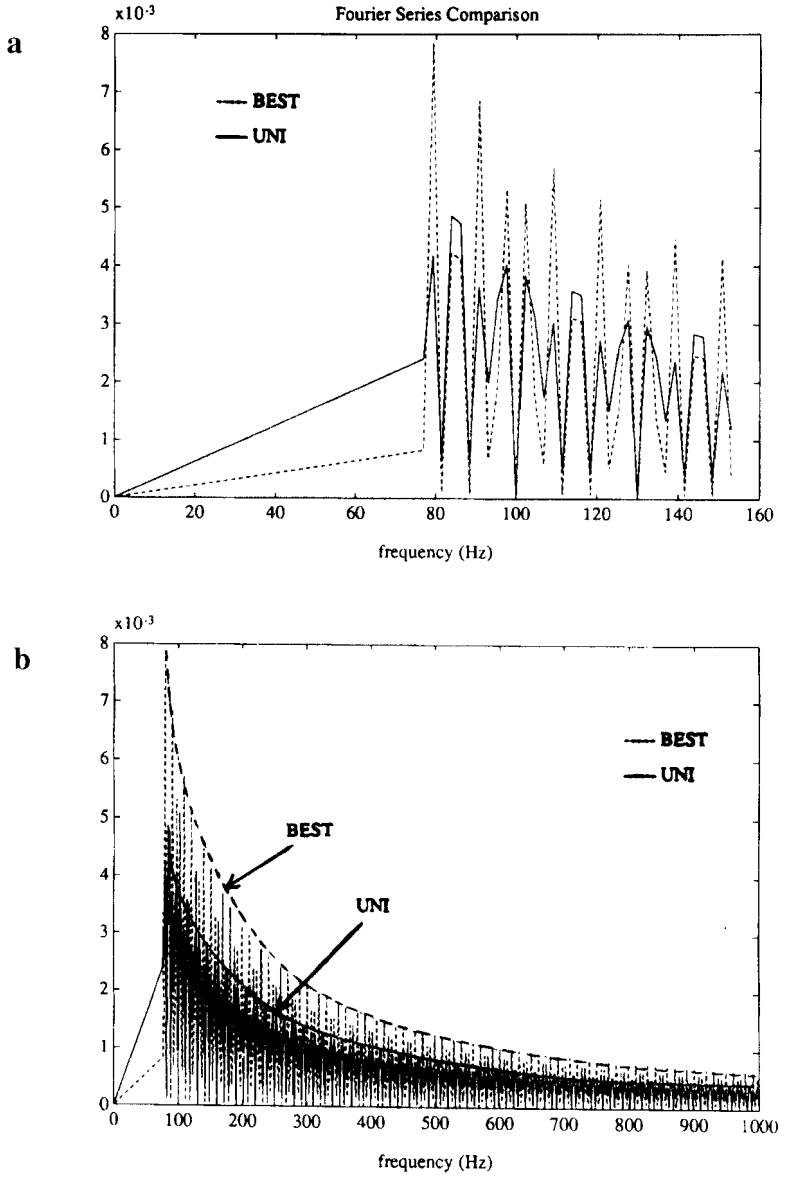

Fig. 4. These spectra are obtained by Fourier transforms of the induced electric field wave shapes. The vertical axis shows the amplitude in volts. BEST, bidirectional electromagnetic stimulation technique; UNI, unidirectional electromagnetic stimulation technique. **a:** Comparison of the two methods up to 160 Hz. **b:** Comparison of the two methods up to 1000 Hz. The envelope of the frequency spectrum of the unidirectional (solid lines) and bidirectional (dashed lines) is shown.

Cell Culture

Chicken tendon fibroblasts were obtained by digestion of 17 day embryonic chick tendons with trypsin and crude bacterial collagenase. The cells were plated at 0.5×10^6 cells/culture dish (~ 35 mm diameter) and grown in 3 ml of Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum, 100 units of penicillin/ml, and 100 mg of streptomycin/ml.

Three identical incubators (Forma Model 3158) were used, each with dummy (control), unidirectional, or bidirectional magnetic induction device. The first 24 h after seeding, no magnetic induction was applied to the cell cultures. The magnetic induction devices were turned on after 24 h. Figure 2 shows the experimental setup. The tissue culture plates are stacked up on a Plexiglas stand which was located inside the electromagnet. The height of the Dulbecco's modified Eagle's medium was 3.1 mm in each tissue culture dish. We did not measure the orientation or the intensity of the earth's magnetic field.

Temperature was constantly monitored in the incubators (37° ± 0.1 °C). The temperature measurement experiments with a very sensitive resistive-temperature probe without tissue cultures but energized magnetic coils did not show any significant temperature variations (0.1 °C) in the incubators. The temperature in the tissue culture plates due to applied PEMFs was not monitored. Previous experiments with similar wave shape, frequency, and amplitude (unidirectional) did not cause any temperature change in the culture medium [Cadossi et al., 1985]. Others have predicted negligible heating due to a typical PEMF field in a tissue culture dish [Parkinson, 1985].

DNA Production

Cell proliferation was determined by calculating the amount of DNA in the culture plates [Erwin et al., 1981]. The amount of DNA was divided by 10 picograms per cell (Berg, unpublished data) to calculate the number of fibroblasts present in the culture plates.

Collagen Production

Collagen production was measured by two assays described by Berg [1982] that involve the measurement of 4-hydroxyproline, an amino acid that is unique to collagen and a few other proteins. Collagen content is calculated from the hydroxyproline measurements.

Collagen Synthesis

Collagen synthesis was measured by labeling cultures with 1 μci/ml [^{14}C] proline for 6 h or 24 h followed by assay of 4-hydroxy [^{14}C] proline. The labeled hydroxyproline is converted to labeled pyrole which is quantified using a scintillation counter [Berg, 1982]. Unlabeled hydroxyproline is added to each sample prior to the assay, and its recovery is determined in order to correct the values obtained for the 4-hydroxy [^{14}C] proline as described by Berg [1982].

Intracellular Cyclic AMP

The intracellular cyclic AMP levels were measured as described by Murray and Farndale [1985] with a slight modification. The cyclic AMP Assay Kit was obtained from Amersham (Buckinghamshire, England). The assay is based on a competition between unlabeled cyclic AMP (cAMP) and a fixed quantity of [^{3}H]cAMP

to complex with a cAMP specific binding protein. The amount of [^{3}H]cAMP-protein complex formed is inversely related to the amount of unlabeled cAMP present in the sample. A standard curve was always created together with the unknown samples to eliminate major errors in the measurement.

Tissue Culture Experiments

A total of five independent experiments were performed. Each experimental point reported in this study represents a measurement that is an average of three culture plates. The semi-circular coil allowed the accommodation of enough samples to be stimulated simultaneously for each experiment (Fig. 2).

Experiment 0. Three identical incubators were tested without any coils in order to ensure that there was no difference in the incubators. Cells were grown for 3 and 5 days in each of the incubators, and cell growth was monitored by measuring DNA accumulation. The results were analyzed with a *t*-test.

Experiment 1. This experiment was designed to understand the effect of electromagnetic stimulation on the initial growth phase of the cell proliferation. Cells were cultured for 4 days. Culture plates were stocked in each of the coils (control, unidirectional, and bidirectional). Every 24 h, the total DNA production was measured and used to calculate cell numbers at that time in each incubator.

Experiment 2. This experiment was designed to understand the effect of electrical stimulation on cell growth up to confluence. In addition, the total collagen content, collagen synthesis, and cAMP were measured at the confluency state. Cells were cultured for 8 days to develop a growth curve. Also, the cells grown for 8 days were examined for collagen content for 8 day period. Collagen synthesis for 24 h on the eight day and intracellular cAMP for 24 h on the eight day were also measured and compared by using a *t*-test.

Experiment 3. This experiment was designed to understand the effect of electrical stimulation on the cell growth up to confluence. Cells were cultured for 7 days to develop a growth curve. The collagen content was measured at 7 days, and collagen synthesis was measured during the last 6 h of the seventh day.

Experiment 4. This experiment was designed to understand the effect of electrical stimulation on the initial phase of the cell growth with (similar to experiment 1) and without bovine serum. Cells were cultured for 4 days. All cells were grown for 2 days in the presence of serum. Then the medium was changed and one set of the cultures was grown for days 3 and 4 without serum in the tissue culture media and the second set of cultures was grown with serum up to the fourth day. The collagen content and collagen synthesis for the last 6 h of days 2, 3, and 4 were measured both with and without serum.

Growth curves were used to calculate doubling time [Dawes, 1972]. The cell proliferation data of all three groups (control, unidirectional, and bidirectional) for all the experiments (1, 2, 3, and 4 with serum) were analyzed for days 1–2, 2–3, and 3–4. An analysis of variance where the experiment is used as a repeated measure (ANOVA-SAS statistical package) was used. The interaction term (day * treatment) which is related to the slope of the growth curve was also examined.

RESULTS

The intensity of the magnetic induction (B field) which was generated perpendicular to the base of the culture plate was measured with a search coil of a

diameter 0.53 cm and 100 turns in the unidirectional and bidirectional coils where the tissue culture plates were located (Fig. 2). The results showed that the magnetic induction (B field) generated by the coils was quite uniform (±8% variation in the B field) (parallel to the B1-A1, B2-A2, etc., lines). A spatial average value of the magnetic induction for unidirectional was 20.33×10^{-4} Tesla and for bidirectional was 21.45×10^{-4} Tesla. The difference in average values was small compared with spatial variations and is inherent in the transducer design. This measurement showed that any possible non-uniform magnetic field (B) stimulation was negligible. The induced electric field (E) due to magnetic induction field lines was a circular pattern in the plane of the tissue culture plate bases (in the horizontal planes 1-1, 2-2, etc.). If classical formulations are used to obtain induced electric fields (E) from the time-dependent magnetic fields [Pilla et al., 1983], the electric field intensities at the edge of the tissue culture plates for unidirectional and bidirectional stimulators are found to be 30.2 and 28.6 μv/cm, respectively. The electric field in this setup is zero at the center and rises to the edge of the tissue culture plates. These values were computed from Figure 3b,d for the negative part of the induced electric field (V_1).

DNA Production

The results of experiment 0 indicated that no statistical difference of cell growth among the incubators at 3 or 5 days existed. A typical cell growth curve is shown in Figure 5 (experiment 3). The calculated doubling times [Dawes, 1972] (t_d in h) of the cells grown in serum are shown in Table 1A. Table 1B shows t_d for cells grown without serum, days 2–3 and 3–4 (from part of experiment 4). Statistical analysis showed that there is no statistical difference in cell proliferation in serum among the groups for days 1–2. However, during the log phase of the growth—that is, days 2–3 (with serum)—there was a statistically significant ($P < 0.006$) interaction term (day * treatment) between the control and the electrically stimulated groups. There were no differences between unidirectional and bidirectional electrically stimulated groups for days 2–3. These two groups have higher DNA synthesis and therefore shorter doubling times than the controls for all experiments. This can be seen in Table 1A.

Cell proliferation data with serum for days 3–4 showed that in the electrically stimulated cultures the DNA synthesis slowed down; hence, their doubling time increased compared to controls (Table 1A). Similar behavior for days 2–3 and 3–4 is observed for the cells grown without serum (Table 1B). Experiments 2 and 3 showed that when the cells are completely confluent at the end of seventh or eighth days, there is no statistical difference on the total count of cells among the groups (Fig. 5).

Collagen Production Synthesis and cAMP Measurements

Matrix synthesis for experiment 2 (cells grown for 8 days) is summarized in Figure 6. There were no statistical differences among any of the groups for collagen production after 8 days as for collagen synthesis or cAMP on the eighth day. Matrix synthesis for experiment 3 (cells grown for 7 days) is summarized in Figure 7. The results showed that the collagen production for 7 days (Fig. 7A) and collagen synthesis for the last 6 h of the seventh day (Fig. 7B) were not different in stimulated cultures as compared with controls. As seen in Figure 8, experiment

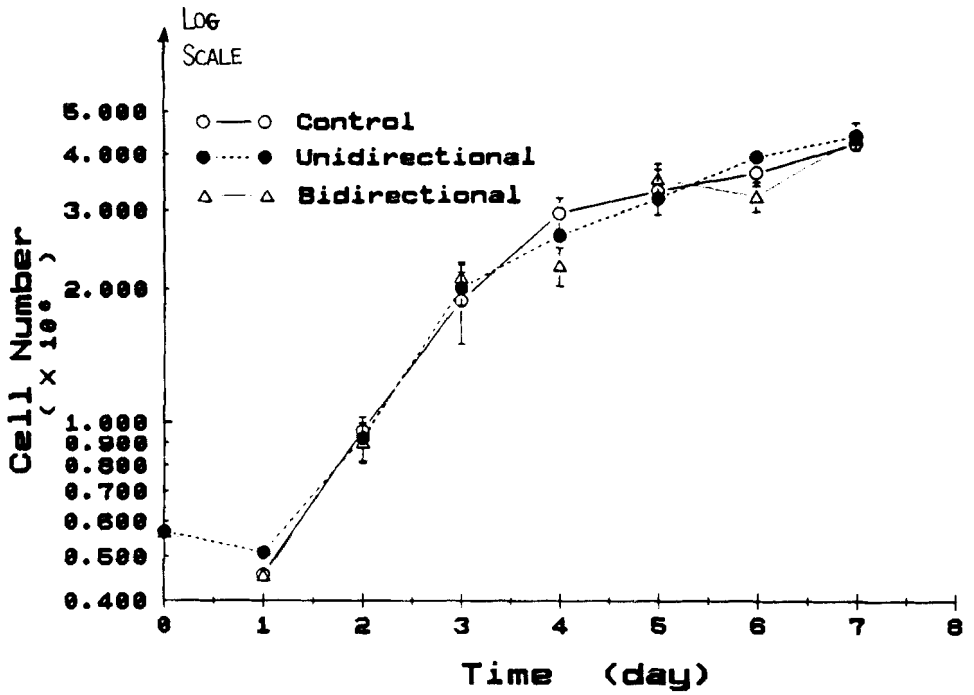

Fig. 5. Cell proliferation for cells cultured 7 days (experiment 3) in log scale. Freshly isolated chick tendon cells were plated as described in Materials and Methods and placed for incubations with a dummy coil, a coil producing a unidirectional magnetic field, and a coil producing a bidirectional magnetic field. The coils were activated at 24 h past plating. The plating efficiency as measured by the number of cells remaining at day 1 compared to the number of cells at day 0 was 80% (control), 90% (unidirectional), and 80% (bidirectional). Bars represent the standard error.

4 showed (Fig. 8) that the collagen content and synthesis were higher per cell on day 2 compared with days 3 and 4 for all groups ($P < 0.018$). The results showed that the collagen content was increased on day 4 compared with day 3 in cultures exposed to the bidirectional ($P < 0.015$) and unidirectional (trend) field with or without serum. Collagen synthesis was decreased at day 4 as compared to day 3 for controls ($P < 0.018$ with serum, trend without serum) and in electrically stimulated cultures (trend) as seen in Figure 8B except for the cultures without serum that were stimulated with the bidirectional field ($P < 0.007$). The collagen synthesis for the unidirectional field at day 2 was lower (trend) than both the controls ($P < 0.053$) and the bidirectional field ($P < 0.064$). In days 3 and 4, collagen content and synthesis were higher in cells for all groups when grown in serum compared with cells cultured without serum (Fig. 8A,B).

DISCUSSION

Cultured fibroblasts have been used to measure the effects of electrical stimulation on cell proliferation and matrix synthesis [Liboff et al., 1984; Murray and Farndale, 1985; Bourguignon and Bourguignon, 1987; McLeod et al., 1987; Murray et al., 1988]. Our results indicate that the final cell density, collagen content per cell, and collagen synthesis per cell at the confluent stage were the same for cul-

TABLE 1. Computed Doubling Time (t_d) in Hours Up to 4 Days for All Experimental Groups (1, 2, 3, and 4)*

		Days		
A: Cells cultured in serum		1–2	2–3	3–4
Experiment 1	Control	15.7	23.3	32.6
	Unidirectional	26.2	16.4	45.3
	Bidirectional	23.0	17.0	50.7
Experiment 2	Control	23.0	20.7	30.2
	Unidirectional	20.1	14.8	44.9
	Bidirectional	22.0	17.0	59.4
Experiment 3	Control	22.4	24.7	37.2
	Unidirectional	28.1	21.4	62.0
	Bidirectional	24.3	19.5	295.0
Part of Experiment 4	Control	51.7	15.2	17.8
	Unidirectional	44.3	12.7	23.9
	Bidirectional	46.0	12.4	50.6
Average of all groups	Control	28.2 (8.0)	21.0 (2.1)	29.4 (4.1)
	Unidirectional	29.7 (5.2)	16.3 (1.85)	44.0 (7.8)
	Bidirectional	28.8 (5.7)	16.5 (1.48)	114.0 (60.4)
B: Cells cultured without serum (from experiment 4).				
Control		51.7	29.7	15.1
Unidirectional		44.3	27.6	15.7
Bidirectional		46.0	19.1	38.9

*Experiment 1 represents the data from 4 day cultures; experiment 2 represents data from 8 day cultures; experiment 3 represents data from 7 day cultures (Fig. 5); and experiment represents data from the experiment for 4 days with and without serum. Doubling time t_d is computed from cell proliferation data of experiments 1, 2, 3, and 4 by using the formula [Dawes, 1972] $t_d = t.\log 2/(\log n - \log n_0)$ where t_d, t, and n stand for doubling time, time taken for population to increase exponentially from n_0 to n (in our case 24 h), and population of cells at any given day, respectively, in our calculations n_0 and n were obtained from DNA measurements for each day in each experiment. They are the average values of three measurements as mentioned in the Materials and Methods section. ANOVA is performed on the calculated t_d values of four experimental measurements. Parentheses show the standard error. They are computed from this table for all experimental groups.

tures with or without electrical stimulation and that there was no difference between unidirectional or bidirectional electrical field stimulated cells.

Murray and Farndale [1985] observed similar results when they cultured chick tendon fibroblast cells and stimulated the cultures with a different PEMF system. They did not observe significant differences in the final cell density at confluency (7 days) between the controls and electrically stimulated cells.

Cell proliferation data (t_d doubling time, Table 1A) for days 3–4 show that in the electrically stimulated cultures DNA synthesis slowed down; hence, their doubling time increased compared with controls. A likely explanation is that electrical stimulation increases cell proliferation at the onset of growth (days 2–3) and, therefore, the electrically stimulated cultures approach confluence more rapidly than controls. When the electrically stimulated cells stop dividing and fill the plate to confluency, the DNA production slows down (days 3–4) as compared to controls. This result shows that the effect of either uni- or bidirectional PEMF stimulation was observed

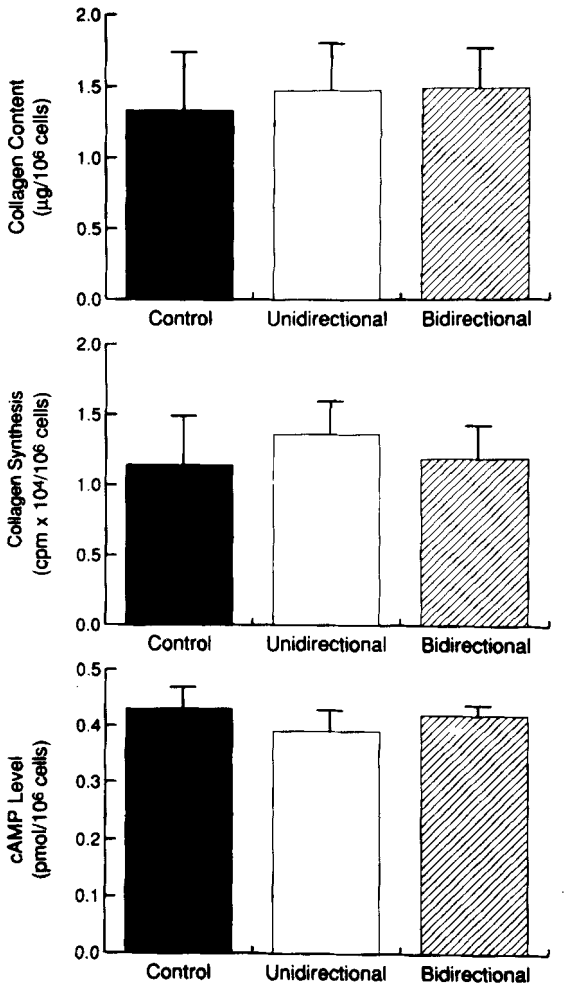

Fig. 6. Collagen content, collagen synthesis, and intracellular cAMP levels for cells grown for 8 days (experiment 2). Collagen synthesis and cAMP levels were measured for 24 h during the eighth day. Bars represent standard deviations. cpm, count per million [Berg, 1982].

at the onset of cell division (Table 1A; t_d for days 2–3). This might suggest that PEMF stimulation could be effective at the onset of fresh structures or wounds, when cell division is rapid. Thus, PEMF stimulation may increase cellular activity and shorten the healing time of fresh fractures [Wahlstrom, 1984] or wounds.

In support of these observations, animal experiments [Goh et al., 1988; Chakkalakal et al., 1990] have shown that new bone formation in a PEMF stimulated group is more rapid than in the control groups during the first half of the fracture healing process. Since fibroblasts are involved in some aspects of soft tissue healing, these experiments suggest that electrical stimulation may have a beneficial effect on wound healing in soft tissues [Jeran et al., 1987; Ieran et al., 1990]. Other researchers using different electromagnetic stimulation techniques have also observed an increased DNA synthesis in cultures exposed to electric fields [Liboff et al., 1984; Norton, 1985; Noda et al., 1987].

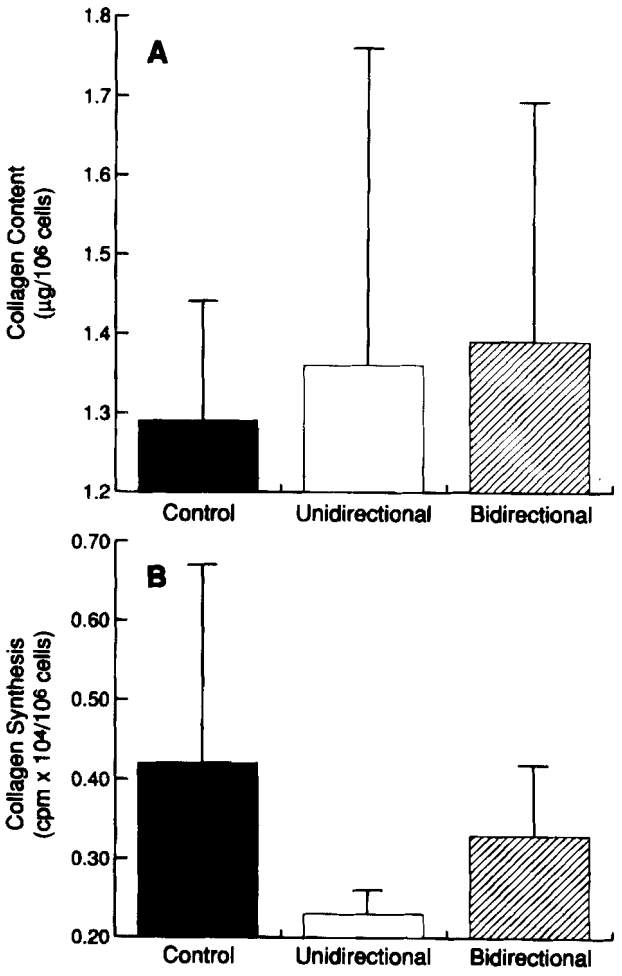

Fig. 7. Collagen content and collagen synthesis for cells grown for 7 days (experiment 3), see Figure 5. Collagen synthesis was measured for the last 6 h of the seventh day. Bars represent standard deviations. No statistical difference was found among any groups for collagen content and synthesis.

Even in the absence of serum, there was an effect of electrical stimulation or cell growth between the second and third day as compared with the control cultures as seen in Table 1B. Serum is known to have a stimulating effect on cell proliferation because of the presence of cell growth factors. The cell growth without serum is slower than in cultures with serum in both controls and PEMF stimulated groups, as shown in Table 1.

The data showed that in any group of cultures grown with or without serum (experiment 4; Fig. 8), in control cultures or in cultures stimulated with unidirectional or bidirectional electrical fields, DNA synthesis was inversely proportional to the collagen synthesis. Therefore, these experiments indicated that collagen synthesis appeared to decrease as cell proliferation increased (Fig. 8). This phenomenon of cellular production of collagen decreasing with increasing DNA synthesis has been observed by other researchers [Norton, 1985; Stein et al., 1989].

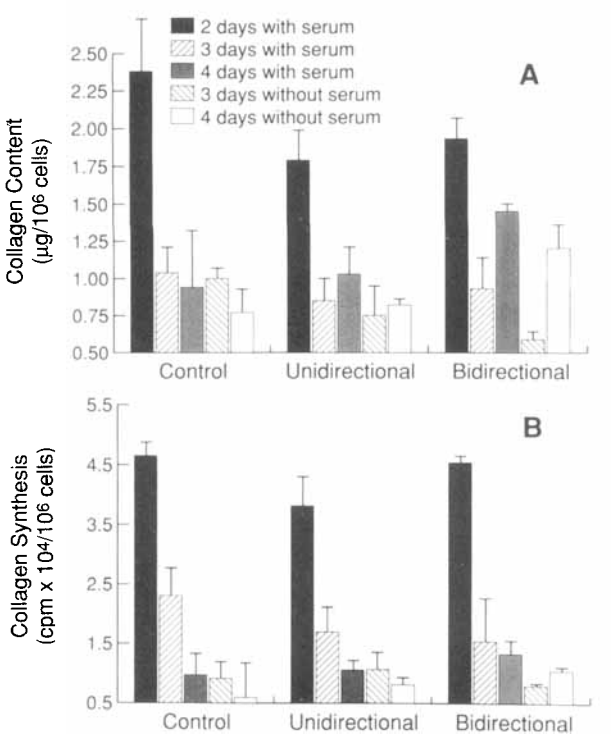

Fig. 8. Collagen content and collagen synthesis for cells grown with or without serum (experiment 4). Collagen synthesis was measured for the last 6 h of days 2, 3, and 4. Bars represent standard deviations. Collagen content and synthesis were higher per cell on day 2 compared with days 3 and 4 for all groups ($P \leq 0.018$). In days 3 and 4, collagen content and synthesis were higher in cells for all groups when the group in serum was compared with cells cultured without serum ($P \leq 0.005$) (trend collagen content: control, unidirectional day 3. Collagen synthesis; control day 4 Unidirectional day 4). Collagen content was increased on day 4 compared with day 3 in cultures exposed to the bidirectional ($P \leq 0.015$) and unidirectional (trend) field with or without serum. Collagen synthesis was decreased at day 4 as compared to day 3 for controls ($P \leq 0.015$ with serum, trend without serum). Collagen synthesis decreased in electrically stimulated groups (trend) except in cultures without serum in the bidirectional field ($P \leq 0.007$).

The experiments reported here did not confirm Murray and Farndale's [1985] experiments in terms of collagen production, collagen synthesis, and cAMP intracellular levels. Murray and Farndale reported that collagen production increased on days 6–7 and 7–8, and cAMP levels decreased in the electrically stimulated group at the end of the sixth day. Other investigators have shown that a change in cAMP occurs after short term electrical stimulation [Norton et al., 1977; Binderman et al., 1985; Brighton and Townsend, 1988]. In our studies, we did not observe any cAMP changes in the confluent state. Murray and Farndale [1985] observed an increase in collagen synthesis at the 6th day in the stimulated groups. Although these authors used the same cell system, cultures of chick tendon fibroblasts, they used a different PEMF system in their experiments.

Therefore, the difference could be due to physical parameters of the applied magnetic field and the spectral content of the employed signal. The spectral content of the signals has been addressed in a discussion by McLeod and Rubin [1990]

in which they attempt to explain some of the experimental results [Rubin et al., 1989]. According to them the power spectral analysis of different wave forms of their electromagnetic stimulation on a turkey ulna model indicated that the different energy levels induced in the tissue at frequencies below 75 Hz are mainly responsible for bone remodeling. In our experiments, the frequency spectra of both electrical stimulation techniques, as shown in Figure 4, generated similar response in DNA production, collagen production, and collagen synthesis. Our frequency spectra below 76.9 Hz has a triangular shape spectra for both uni- and bidirectional electrical stimulation techniques, though unidirectional has a larger area. In our experiments, both signals generated similar biological responses; therefore, in order to explain our results with a similar energy input criteria as suggested by McLeod and Rubin [1990] for our experimental model, we have to take into account frequencies higher than 76.9 for the bidirectional field.

In sum:

1. PEMF stimulation is effective at the onset of cell growth in log phase by decreasing doubling time (t_d), thus increasing cell division as measured by DNA synthesis.

2. When cells are dividing more rapidly, collagen synthesis is reduced. In the confluent state, there were no differences among any groups in terms of collagen synthesis and cAMP production.

3. When tissue cultures reach confluency, there is no difference among the groups in cell density. The results here suggest that the effect of either uni- or bidirectional PEMF electrical stimulation can be seen at the onset of cell division.

4. The two different wave shapes (unidirectional and bidirectional) employing similar repetition rate, duty cycle, and total amplitude generated similar responses in tissue culture experiments. In general the effect of PEMF systems does not appear to depend strongly on the magnetic polarity of wave shapes. The effects might, however, depend on other parameters such as repetition rate, duty cycle, total amplitude, and frequency spectrum.

5. This tissue culture experiment suggests that if the energy efficient design technique is used in addition to the energy recovery, it is possible to develop a smaller PEMF stimulator for therapeutic applications.

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