

ELECTROMAGNETIC TREATMENT APPARATUS AND METHOD**TECHNICAL FIELD**

5 This invention pertains generally to an apparatus and a method for treatment of living tissues and cells by altering their interaction with their electromagnetic environment. This invention also relates to a method of modification of cellular and tissue growth, repair, maintenance, and general behavior by application of encoded electromagnetic information. More particularly this invention relates to the application of surgically non-invasive coupling of highly specific electromagnetic signal patterns to any number of body parts. In particular, an embodiment according to the present invention pertains to using pulsing electromagnetic fields ("PEMF") to enhance living tissue growth and repair via angiogenesis and neovascularization by affecting the precursors to growth factors and other cytokines, such as ion/ligand binding such as calcium binding to calmodoulin.

BACKGROUND ART

It is now well established that application of weak non-thermal electromagnetic fields ("EMF") can result in physiologically meaningful in vivo and in vitro bioeffects.

EMF has been used in applications of bone repair and bone healing. Waveforms comprising low frequency components and low power are currently used in orthopedic clinics. Origins of using bone repair signals began by considering that an electrical pathway may constitute a means through which bone can adaptively respond to EMF signals. A linear physicochemical approach employing an electrochemical model of a cell membrane predicted a range of EMF waveform patterns for which bioeffects might be expected. Since a cell membrane was a likely EMF target, it

became necessary to find a range of waveform parameters for which an induced electric field could couple electrochemically at the cellular surface, such as voltage-dependent kinetics. Extension of this linear model also involved Lorentz force analysis.

A pulsed radio frequency ("PRF") signal derived from a 27.12 MHz continuous sine wave used for deep tissue healing is known in the prior art of diathermy. A pulsed successor of the diathermy signal was originally reported as an electromagnetic field capable of eliciting a non-thermal biological effect in the treatment of infections. PRF therapeutic applications have been reported for reduction of post-traumatic and post-operative pain and edema in soft tissues, wound healing, burn treatment and nerve regeneration. Application of EMF for the resolution of traumatic edema has become increasingly used in recent years. Results to date using PRF in animal and clinical studies suggest that edema may be measurably reduced from such electromagnetic stimulus.

Prior art considerations of EMF dosimetry have not taken into account dielectric properties of tissue structure as opposed to the properties of isolated cells.

In recent years, clinical use of non-invasive PRF at radio frequencies comprised using pulsed bursts of a 27.12 MHz sinusoidal wave, wherein each pulse burst comprises a width of sixty-five microseconds, having approximately 1,700 sinusoidal cycles per burst, and various burst repetition rates. By use of a substantially single voltage amplitude envelope with each PRF burst, one was limiting frequency components that could couple to relevant dielectric pathways in cells and tissue.

Time-varying electromagnetic fields, comprising rectangular waveforms such as pulsing electromagnetic fields, and sinusoidal waveforms such as pulsed radio frequency fields ranging from several Hertz to an about 15 to an about 40 MHz

range, are clinically beneficial when used as an adjunctive therapy for a variety of musculoskeletal injuries and conditions.

Beginning in the 1960's, development of modern therapeutic and prophylactic devices was stimulated by clinical problems associated with non-union and delayed union bone fractures. Early work showed that an electrical pathway can be a means through which bone adaptively responds to mechanical input. Early therapeutic devices used implanted and semi-invasive electrodes delivering direct current ("DC") to a fracture site. Non-invasive technologies were subsequently developed using electrical and electromagnetic fields. These modalities were originally created to provide a non-invasive "no-touch" means of inducing an electrical/mechanical waveform at a cell/tissue level. Clinical applications of these technologies in orthopaedics have led to approved applications by regulatory bodies worldwide for treatment of fractures such as non-unions and fresh fractures, as well as spine fusion. Presently several EMF devices constitute the standard armamentarium of orthopaedic clinical practice for treatment of difficult to heal fractures. The success rate for these devices has been very high. The database for this indication is large enough to enable its recommended use as a safe, non-surgical, non-invasive alternative to a first bone graft. Additional clinical indications for these technologies have been reported in double blind studies for treatment of avascular necrosis, tendinitis, osteoarthritis, wound repair, blood circulation and pain from arthritis as well as other musculoskeletal injuries.

Cellular studies have addressed effects of weak low frequency electromagnetic fields on both signal transduction pathways and growth factor synthesis. It can be shown that EMF stimulates secretion of growth factors after a short, trigger-like duration. Ion/ligand binding processes at a cell membrane are generally considered an initial EMF target pathway structure. The clinical relevance to treatments for example of

bone repair, is upregulation such as modulation, of growth factor production as part of normal molecular regulation of bone repair. Cellular level studies have shown effects on calcium ion transport, cell proliferation, Insulin Growth Factor ("IGF-II") release, and IGF-II receptor expression in osteoblasts. Effects on Insulin Growth Factor-I ("IGF-I") and IGF-II have also been demonstrated in rat fracture callus. Stimulation of transforming growth factor beta ("TGF- β ") messenger RNA ("mRNA") with PEMF in a bone induction model in a rat has been shown. Studies have also demonstrated upregulation of TGF- β mRNA by PEMF in human osteoblast-like cell line designated MG-63, wherein there were increases in TGF- β 1, collagen, and osteocalcin synthesis. PEMF stimulated an increase in TGF- β 1 in both hypertrophic and atrophic cells from human non-union tissue. Further studies demonstrated an increase in both TGF- β 1 mRNA and protein in osteoblast cultures resulting from a direct effect of EMF on a calcium/calmodulin-dependent pathway. Cartilage cell studies have shown similar increases in TGF- β 1 mRNA and protein synthesis from EMF, demonstrating a therapeutic application to joint repair. Various studies conclude that upregulation of growth factor production may be a common denominator in the tissue level mechanisms underlying electromagnetic stimulation. When using specific inhibitors, EMF can act through a calmodulin-dependent pathway. It has been previously reported that specific PEMF and PRF signals, as well as weak static magnetic fields, modulate Ca^{2+} binding to CaM in a cell-free enzyme preparation. Additionally, upregulation of mRNA for BMP2 and BMP4 with PEMF in osteoblast cultures and upregulation of TGF- β 1 in bone and cartilage with PEMF have been demonstrated.

However, prior art in this field does not configure waveforms based upon a ion/ligand binding transduction pathway. Prior art waveforms are inefficient since prior art waveforms apply unnecessarily high amplitude and power to living tissues

and cells, require unnecessarily long treatment time, and cannot be generated by a portable device.

Therefore, a need exists for an apparatus and a method that more effectively modulates angiogenesis and other biochemical processes that regulate tissue growth and repair, shortens treatment times, and incorporates miniaturized circuitry and light weight applicators thus allowing the apparatus to be portable and if desired disposable. A further need exists for an apparatus and method that more effectively modulates angiogenesis and other biochemical processes that regulate tissue growth and repair, shortens treatment times, and incorporates miniaturized circuitry and light weight applicators that can be constructed to be implantable.

DISCLOSURE OF INVENTION

An apparatus and a method for electromagnetic treatment of living tissues and cells by altering their interaction with their electromagnetic environment.

According to an embodiment of the present invention, by treating a selectable body region with a flux path comprising a succession of EMF pulses having a minimum width characteristic of at least about 0.01 microseconds in a pulse burst envelope having between about 1 and about 100,000 pulses per burst, in which a voltage amplitude envelope of said pulse burst is defined by a randomly varying parameter in which instantaneous minimum amplitude thereof is not smaller than the maximum amplitude thereof by a factor of one ten-thousandth. The pulse burst repetition rate can vary from about 0.01 to about 10,000 Hz. A mathematically definable parameter can also be employed to define an amplitude envelope of said pulse bursts.

By increasing a range of frequency components transmitted to relevant cellular pathways, access to a large range of biophysical phenomena applicable to known healing mechanisms,

including enhanced enzyme activity and growth factor and cytokine release, is advantageously achieved.

According to an embodiment of the present invention, by applying a random, or other high spectral density envelope, to a pulse burst envelope of mono- or bi-polar rectangular or sinusoidal pulses which induce peak electric fields between 10^{-6} and 10 volts per centimeter (V/cm), a more efficient and greater effect can be achieved on biological healing processes applicable to both soft and hard tissues in humans, animals and plants. A pulse burst envelope of higher spectral density can advantageously and efficiently couple to physiologically relevant dielectric pathways, such as, cellular membrane receptors, ion binding to cellular enzymes, and general transmembrane potential changes thereby modulating angiogenesis and neovascularization.

By advantageously applying a high spectral density voltage envelope as a modulating or pulse-burst defining parameter, power requirements for such modulated pulse bursts can be significantly lower than that of an unmodulated pulse. This is due to more efficient matching of the frequency components to the relevant cellular/molecular process. Accordingly, the dual advantages of enhanced transmitting dosimetry to relevant dielectric pathways and of decreasing power requirements are achieved.

A preferred embodiment according to the present invention utilizes a Power Signal to Noise Ratio ("Power SNR") approach to configure bioeffective waveforms and incorporates miniaturized circuitry and lightweight flexible coils. This advantageously allows a device that utilizes a Power SNR approach, miniaturized circuitry, and lightweight flexible coils, to be completely portable and if desired to be constructed as disposable and if further desired to be constructed as implantable.

Specifically, broad spectral density bursts of electromagnetic waveforms, configured to achieve maximum signal

power within a bandpass of a biological target, are selectively applied to target pathway structures such as living organs, tissues, cells and molecules. Waveforms are selected using a unique amplitude/power comparison with that of thermal noise in a target pathway structure. Signals comprise bursts of at least one of sinusoidal, rectangular, chaotic and random wave shapes, have frequency content in a range of about 0.01 Hz to about 100 MHz at about 1 to about 100,000 bursts per second, and have a burst repetition rate from about 0.01 to about 1000 bursts/second. Peak signal amplitude at a target pathway structure such as tissue, lies in a range of about 1 μ V/cm to about 100 mV/cm. Each signal burst envelope may be a random function providing a means to accommodate different electromagnetic characteristics of healing tissue. A preferred embodiment according to the present invention comprises about 0.1 to about 100 millisecond pulse burst comprising about 1 to about 200 microsecond symmetrical or asymmetrical pulses repeating at about 0.1 to about 100 kilohertz within the burst. The burst envelope is a modified 1/f function and is applied at random repetition rates between about 0.1 Hz and about 1000 Hz. Fixed repetition rates can also be used between about 0.1 Hz and about 1000 Hz. An induced electric field from about 0.001 mV/cm to about 100 mV/cm is generated. Another embodiment according to the present invention comprises an about 0.01 millisecond to an about 10 millisecond burst of high frequency sinusoidal waves, such as 27.12 MHz, repeating at about 1 to about 100 bursts per second. An induced electric field from about 0.001 mV/cm to about 100 mV/cm is generated. Resulting waveforms can be delivered via inductive or capacitive coupling.

It is an object of the present invention to provide modulation of electromagnetically sensitive regulatory processes at the cell membrane and at junctional interfaces between cells.

It is another object of the present invention to provide an electromagnetic method of treatment of living cells and tissues comprising a broad-band, high spectral density electromagnetic field.

5 It is a further object of the present invention to provide an electromagnetic method of treatment of living cells and tissues comprising amplitude modulation of a pulse burst envelope of an electromagnetic signal that will induce coupling with a maximum number of relevant EMF-sensitive pathways in
10 cells or tissues.

It is another object of the present invention to provide increased blood flow to affected tissues by modulating angiogenesis and neovascularization.

15 It is another object of the present invention to provide increased blood flow to enhance viability, growth, and differentiation of implanted cells, such as stem cells, tissues and organs.

20 It is another object of the present invention to provide increased blood flow in cardiovascular diseases by modulating angiogenesis and neovascularization.

It is another object of the present invention to improve micro-vascular blood perfusion and reduced transudation.

25 It is another object of the present invention to provide a treatment of maladies of the bone and other hard tissue by modulating angiogenesis and neovascularization.

It is a still further object of the present invention to provide a treatment of edema and swelling of soft tissue by increased blood flow through modulation of angiogenesis and neovascularization.

It is another object of the present invention to provide an electromagnetic method of treatment of living cells and tissues that can be used for repair of damaged soft tissue.

It is yet another object of the present invention to increase blood flow to damaged tissue by modulation of vasodilation and stimulating neovascularization.

It is a yet further object of the present invention to provide an apparatus for modulation of angiogenesis and neovascularization that can be operated at reduced power levels and still possess benefits of safety, economics, portability, and reduced electromagnetic interference.

It is an object of the present invention to configure a power spectrum of a waveform by mathematical simulation by using signal to noise ratio ("SNR") analysis to configure a waveform optimized to modulate angiogenesis and neovascularization then coupling the configured waveform using a generating device such as ultra lightweight wire coils that are powered by a waveform configuration device such as miniaturized electronic circuitry.

It is another object of the present invention to modulate angiogenesis and neovascularization by evaluating Power SNR for any target pathway structure such as molecules, cells, tissues and organs of plants, animals and humans using any input waveform, even if electrical equivalents are non-linear as in a Hodgkin-Huxley membrane model.

It is another object of the present invention to provide a method and apparatus for treating plants, animals and humans using electromagnetic fields selected by optimizing a power spectrum of a waveform to be applied to a biochemical target pathway structure to enable modulation of angiogenesis and neovascularization within molecules, cells, tissues and organs of a plant, animal, and human.

It is another object of the present invention to significantly lower peak amplitudes and shorter pulse duration. This can be accomplished by matching via Power SNR, a frequency

range in a signal to frequency response and sensitivity of a target pathway structure such as a molecule, cell, tissue, and organ, of plants, animals and humans to enable modulation of angiogenesis and neovascularization.

5 The above and yet other objects and advantages of the present invention will become apparent from the hereinafter set forth Brief Description of the Drawings, Detailed Description of the Invention, and Claims appended herewith.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the present invention will be described below in more detail, with reference to the accompanying drawings:

15 Figure 1 is a flow diagram of a method for electromagnetic treatment of living tissues and cells according to an embodiment of the present invention;

Figure 2 is a view of control circuitry ----- according to a preferred embodiment of the present invention;

20 Figure 3 is a block diagram of miniaturized circuitry according to a preferred embodiment of the present invention;

Figure 4 depicts a waveform delivered to a target pathway structure such as a molecule, cell, tissue or organ according to a preferred embodiment of the present invention.

MODES FOR CARRYING OUT THE INVENTION

25 Induced time-varying currents from PEMF or PRF devices flow in a target pathway structure such as a molecule, cell, tissue, and organ, and it is these currents that are a stimulus to which cells and tissues can react in a physiologically meaningful manner. The electrical properties of a target pathway structure affect levels and distributions of induced current. Molecules, cells, tissue, and organs are all in an induced current pathway such as cells in a gap junction contact. Ion or ligand

interactions at binding sites on macromolecules that may reside on a membrane surface are voltage dependent processes, that is electrochemical, that can respond to an induced electromagnetic field ("E"). Induced current arrives at these sites via a surrounding ionic medium. The presence of cells in a current pathway causes an induced current ("J") to decay more rapidly with time ("J(t)"). This is due to an added electrical impedance of cells from membrane capacitance and time constants of binding and other voltage sensitive membrane processes such as membrane transport.

Equivalent electrical circuit models representing various membrane and charged interface configurations have been derived. For example, in Calcium ("Ca²⁺") binding, the change in concentration of bound Ca²⁺ at a binding site due to induced E may be described in a frequency domain by an impedance expression such as:

$$Z_b(\omega) = R_{ion} + \frac{1}{i\omega C_{ion}}$$

which has the form of a series resistance-capacitance electrical equivalent circuit. Where ω is angular frequency defined as $2\pi f$, where f is frequency, $i = -1^{1/2}$, $Z_b(\omega)$ is the binding impedance, and R_{ion} and C_{ion} are equivalent binding resistance and capacitance of an ion binding pathway. The value of the equivalent binding time constant, $\tau_{ion} = R_{ion}C_{ion}$, is related to a ion binding rate constant, k_b , via $\tau_{ion} = R_{ion}C_{ion} = 1/k_b$. Thus, the characteristic time constant of this pathway is determined by ion binding kinetics.

Induced E from a PEMF or PRF signal can cause current to flow into an ion binding pathway and affect the number of Ca²⁺ ions bound per unit time. An electrical equivalent of this is a change in voltage across the equivalent binding capacitance C_{ion} , which is a direct measure of the change in electrical charge

stored by C_{ion} . Electrical charge is directly proportional to a surface concentration of Ca^{2+} ions in the binding site, that is storage of charge is equivalent to storage of ions or other charged species on cell surfaces and junctions. Electrical impedance measurements, as well as direct kinetic analyses of binding rate constants, provide values for time constants necessary for configuration of a PMF waveform to match a bandpass of target pathway structures. This allows for a required range of frequencies for any given induced E waveform for optimal coupling to target impedance, such as bandpass.

Ion binding to regulatory molecules is a frequent EMF target, for example Ca^{2+} binding to calmodulin ("CaM"). Use of this pathway is based upon acceleration of wound repair, for example bone repair, that involves modulation of growth factors released in various stages of repair. Growth factors such as platelet derived growth factor ("PDGF"), fibroblast growth factor ("FGF"), and epidermal growth factor ("EGF") are all involved at an appropriate stage of healing. Angiogenesis and neovascularization are also integral to wound repair and can be modulated by PMF. All of these factors are Ca/CaM-dependent.

Utilizing a Ca/CaM pathway a waveform can be configured for which induced power is sufficiently above background thermal noise power. Under correct physiological conditions, this waveform can have a physiologically significant bioeffect.

Application of a Power SNR model to Ca/CaM requires knowledge of electrical equivalents of Ca^{2+} binding kinetics at CaM. Within first order binding kinetics, changes in concentration of bound Ca^{2+} at CaM binding sites over time may be characterized in a frequency domain by an equivalent binding time constant, $\tau_{ion} = R_{ion}C_{ion}$, where R_{ion} and C_{ion} are equivalent binding resistance and capacitance of the ion binding pathway. τ_{ion} is related to a ion binding rate constant, k_b , via $\tau_{ion} = R_{ion}C_{ion} = 1/k_b$. Published values for k_b can then be employed in a cell array model to evaluate SNR by comparing voltage induced by a PRF signal to thermal fluctuations in voltage at a CaM binding

site. Employing numerical values for PMF response, such as
 $V_{\max}=6.5 \times 10^{-7} \text{ sec}^{-1}$, $[\text{Ca}^{2+}]=2.5 \mu\text{M}$, $K_D=30 \mu\text{M}$,
 $[\text{Ca}^{2+}\text{CaM}]=K_D([\text{Ca}^{2+}]+[\text{CaM}])$, yields $k_b=665 \text{ sec}^{-1}$ ($\tau_{\text{ion}} = 1.5 \text{ msec}$).
 Such a value for τ_{ion} can be employed in an electrical equivalent
 5 circuit for ion binding while power SNR analysis can be
 performed for any waveform structure.

According to an embodiment of the present invention a
 mathematical model can be configured to assimilate that thermal
 noise is present in all voltage dependent processes and represents
 10 a minimum threshold requirement to establish adequate SNR. Power
 spectral density, $S_n(\omega)$, of thermal noise can be expressed as:

$$S_n(\omega) = 4kT \text{Re}[Z_M(x, \omega)]$$

where $Z_M(x, \omega)$ is electrical impedance of a target pathway
 structure, x is a dimension of a target pathway structure and Re
 15 denotes a real part of impedance of a target pathway structure.
 $Z_M(x, \omega)$ can be expressed as:

$$Z_M(x, \omega) = \left[\frac{R_e + R_i + R_g}{\gamma} \right] \tanh(\gamma x)$$

This equation clearly shows that electrical impedance of
 the target pathway structure, and contributions from
 20 extracellular fluid resistance ("R_e"), intracellular fluid
 resistance ("R_i") and intermembrane resistance ("R_g") which are
 electrically connected to a target pathway structure, all
 contribute to noise filtering.

A typical approach to evaluation of SNR uses a single value
 25 of a root mean square (RMS) noise voltage. This is calculated by
 taking a square root of an integration of
 $S_n(\omega) = 4kT \text{Re}[Z_M(x, \omega)]$ over all frequencies relevant to either
 complete membrane response, or to bandwidth of a target pathway
 structure. SNR can be expressed by a ratio:

$$SNR = \frac{|V_M(\omega)|}{RMS}$$

where $|V_M(\omega)|$ is maximum amplitude of voltage at each frequency as delivered by a chosen waveform to the target pathway structure.

An embodiment according to the present invention comprises
5 a pulse burst envelope having a high spectral density, so that the effect of therapy upon the relevant dielectric pathways, such as, cellular membrane receptors, ion binding to cellular enzymes and general transmembrane potential changes, is enhanced. Accordingly by increasing a number of frequency
10 components transmitted to relevant cellular pathways, a large range of biophysical phenomena, such as modulating growth factor and cytokine release and ion binding at regulatory molecules, applicable to known healing mechanisms is accessible. According to an embodiment of the present invention applying a random, or
15 other high spectral density envelope, to a pulse burst envelope of mono- or bi-polar rectangular or sinusoidal pulses inducing peak electric fields between about 10^{-6} and about 100 V/cm, produces a greater effect on biological healing processes applicable to both soft and hard tissues.

20 According to yet another embodiment of the present invention by applying a high spectral density voltage envelope as a modulating or pulse-burst defining parameter, power requirements for such amplitude modulated pulse bursts can be significantly lower than that of an unmodulated pulse burst
25 containing pulses within a similar frequency range. This is due to a substantial reduction in duty cycle within repetitive burst trains brought about by imposition of an irregular, and preferably random, amplitude onto what would otherwise be a substantially uniform pulse burst envelope. Accordingly, the
30 dual advantages, of enhanced transmitted dosimetry to the relevant dielectric pathways and of decreased power requirement are achieved.

Referring to Figure 1, wherein Figure 1 is a flow diagram of a method for delivering electromagnetic signals to angiogenesis and neovascularization target pathway structures such as ions and ligands of plants, animals, and humans for therapeutic and prophylactic purposes according to an embodiment of the present invention. A mathematical model having at least one waveform parameter is applied to configure at least one waveform to be coupled to a angiogenesis and neovascularization target pathway structure such as ions and ligands (Step 101). The configured waveform satisfies a SNR or Power SNR model so that for a given and known angiogenesis and neovascularization target pathway structure it is possible to choose at least one waveform parameter so that a waveform is detectable in the angiogenesis and neovascularization target pathway structure above its background activity (Step 102) such as baseline thermal fluctuations in voltage and electrical impedance at a target pathway structure that depend upon a state of a cell and tissue, that is whether the state is at least one of resting, growing, replacing, and responding to injury. A preferred embodiment of a generated electromagnetic signal is comprised of a burst of arbitrary waveforms having at least one waveform parameter that includes a plurality of frequency components ranging from about 0.01 Hz to about 100 MHz wherein the plurality of frequency components satisfies a Power SNR model (Step 102). A repetitive electromagnetic signal can be generated for example inductively or capacitively, from said configured at least one waveform (Step 103). The electromagnetic signal is coupled to a angiogenesis and neovascularization target pathway structure such as ions and ligands by output of a coupling device such as an electrode or an inductor, placed in close proximity to the target pathway structure (Step 104). The coupling enhances modulation of binding of ions and ligands to regulatory molecule in living tissues and cells.

Figure 2 illustrates a preferred embodiment of an apparatus according to the present invention. A miniature control circuit

201 is coupled to an end of at least one connector 202 such as wire. The opposite end of the at least one connector is coupled to a generating device such as a pair of electrical coils 203. The miniature control circuit 201 is constructed in a manner that applies a mathematical model that is used to configure waveforms. The configured waveforms have to satisfy a SNR or Power SNR model so that for a given and known angiogenesis and neovascularization target pathway structure, it is possible to choose waveform parameters that satisfy SNR or Power SNR so that a waveform is detectable in the angiogenesis and neovascularization target pathway structure above its background activity. A preferred embodiment according to the present invention applies a mathematical model to induce a time-varying magnetic field and a time-varying electric field in a angiogenesis and neovascularization target pathway structure such as ions and ligands, comprising about 10 to about 100 msec bursts of about 1 to about 100 microsecond rectangular pulses repeating at about 0.1 to about 10 pulses per second. Peak amplitude of the induced electric field is between about 1 uV/cm and about 100 mV/cm, varied according to a modified 1/f function where f = frequency. A waveform configured using a preferred embodiment according to the present invention may be applied to a angiogenesis and neovascularization target pathway structure such as ions and ligands for a preferred total exposure time of under 1 minute to 240 minutes daily. However other exposure times can be used. Waveforms configured by the miniature control circuit 201 are directed to a generating device 203 such as electrical coils via connector 202. The generating device 203 delivers a pulsing magnetic field configured according to a mathematical model, that can be used to provide treatment to a angiogenesis and neovascularization target pathway structure such as a heart in a chest 204. The miniature control circuit applies a pulsing magnetic field for a prescribed time and can automatically repeat applying the pulsing magnetic field for as many applications as are needed in a given time period, for

example 10 times a day. A preferred embodiment according to the present invention can be positioned to treat the heart in the chest 204 by a positioning device. Coupling a pulsing magnetic field to a angiogenesis and neovascularization target pathway structure such as ions and ligands, therapeutically and prophylactically reduces inflammation thereby reducing pain and promotes healing. When electrical coils are used as the generating device 203, the electrical coils can be powered with a time varying magnetic field that induces a time varying electric field in a target pathway structure according to Faraday's law. An electromagnetic signal generated by the generating device 203 can also be applied using electrochemical coupling, wherein electrodes are in direct contact with skin or another outer electrically conductive boundary of a target pathway structure. Yet in another embodiment according to the present invention, the electromagnetic signal generated by the generating device 203 can also be applied using electrostatic coupling wherein an air gap exists between a generating device 203 such as an electrode and a angiogenesis and neovascularization target pathway structure such as ions and ligands. An advantage of the preferred embodiment according to the present invention is that its ultra lightweight coils and miniaturized circuitry allow for use with common physical therapy treatment modalities and at any body location for which pain relief and healing is desired. An advantageous result of application of the preferred embodiment according to the present invention is that a living organism's angiogenesis and neovascularization can be maintained and enhanced.

Figure 3 depicts a block diagram of a preferred embodiment according to the present invention of a miniature control circuit 300. The miniature control circuit 300 produces waveforms that drive a generating device such as wire coils described above in Figure 2. The miniature control circuit can be activated by any activation means such as an on/off switch. The miniature control circuit 300 has a power source such as a

lithium battery 301. A preferred embodiment of the power source has an output voltage of 3.3 V but other voltages can be used. In another embodiment according to the present invention the power source can be an external power source such as an electric current outlet such as an AC/DC outlet, coupled to the present invention for example by a plug and wire. A switching power supply 302 controls voltage to a micro-controller 303. A preferred embodiment of the micro-controller 303 uses an 8 bit 4 MHz micro-controller 303 but other bit MHz combination micro-controllers may be used. The switching power supply 302 also delivers current to storage capacitors 304. A preferred embodiment of the present invention uses storage capacitors having a 220 uF output but other outputs can be used. The storage capacitors 304 allow high frequency pulses to be delivered to a coupling device such as inductors (Not Shown). The micro-controller 303 also controls a pulse shaper 305 and a pulse phase timing control 306. The pulse shaper 305 and pulse phase timing control 306 determine pulse shape, burst width, burst envelope shape, and burst repetition rate. An integral waveform generator, such as a sine wave or arbitrary number generator can also be incorporated to provide specific waveforms. A voltage level conversion sub-circuit 308 controls an induced field delivered to a target pathway structure. A switching Hexfet 308 allows pulses of randomized amplitude to be delivered to output 309 that routes a waveform to at least one coupling device such as an inductor. The micro-controller 303 can also control total exposure time of a single treatment of a target pathway structure such as a molecule, cell, tissue, and organ. The miniature control circuit 300 can be constructed to apply a pulsing magnetic field for a prescribed time and to automatically repeat applying the pulsing magnetic field for as many applications as are needed in a given time period, for example 10 times a day. A preferred embodiment according to the present invention uses treatments times of about 10 minutes to about 30 minutes.

Referring to Figure 4 an embodiment according to the present invention of a waveform 400 is illustrated. A pulse 401 is repeated within a burst 402 that has a finite duration 403. The duration 403 is such that a duty cycle which can be defined as a ratio of burst duration to signal period is between about 1 to about 10^{-5} . A preferred embodiment according to the present invention utilizes pseudo rectangular 10 microsecond pulses for pulse 401 applied in a burst 402 for about 10 to about 50 msec having a modified $1/f$ amplitude envelope 404 and with a finite duration 403 corresponding to a burst period of between about 0.1 and about 10 seconds.

Example 1

The Power SNR approach for PMF signal configuration has been tested experimentally on calcium dependent myosin phosphorylation in a standard enzyme assay. The cell-free reaction mixture was chosen for phosphorylation rate to be linear in time for several minutes, and for sub-saturation Ca^{2+} concentration. This opens the biological window for $\text{Ca}^{2+}/\text{CaM}$ to be EMF-sensitive. This system is not responsive to PMF at levels utilized in this study if Ca^{2+} is at saturation levels with respect to CaM, and reaction is not slowed to a minute time range. Experiments were performed using myosin light chain ("MLC") and myosin light chain kinase ("MLCK") isolated from turkey gizzard. A reaction mixture consisted of a basic solution containing 40 mM Hepes buffer, pH 7.0; 0.5 mM magnesium acetate; 1 mg/ml bovine serum albumin, 0.1% (w/v) Tween 80; and 1 mM EGTA12. Free Ca^{2+} was varied in the 1-7 μM range. Once Ca^{2+} buffering was established, freshly prepared 70 nM CaM, 160 nM MLC and 2 nM MLCK were added to the basic solution to form a final reaction mixture. The low MLC/MLCK ratio allowed linear time behavior in the minute time range. This provided reproducible enzyme activities and minimized pipetting time errors.

The reaction mixture was freshly prepared daily for each series of experiments and was aliquoted in 100 μ L portions into 1.5 ml Eppendorf tubes. All Eppendorf tubes containing reaction mixture were kept at 0°C then transferred to a specially designed water bath maintained at $37 \pm 0.1^\circ\text{C}$ by constant perfusion of water prewarmed by passage through a Fisher Scientific model 900 heat exchanger. Temperature was monitored with a thermistor probe such as a Cole-Parmer model 8110-20, immersed in one Eppendorf tube during all experiments. Reaction was initiated with 2.5 μM 32P ATP, and was stopped with Laemmli Sample Buffer solution containing 30 μM EDTA. A minimum of five blank samples were counted in each experiment. Blanks comprised a total assay mixture minus one of the active components Ca^{2+} , CaM, MLC or MLCK. Experiments for which blank counts were higher than 300 cpm were rejected. Phosphorylation was allowed to proceed for 5 min and was evaluated by counting 32P incorporated in MLC using a TM Analytic model 5303 Mark V liquid scintillation counter.

The signal comprised repetitive bursts of a high frequency waveform. Amplitude was maintained constant at 0.2G and repetition rate was 1 burst/sec for all exposures. Burst duration varied from 65 μsec to 1000 μsec based upon projections of Power SNR analysis which showed that optimal Power SNR would be achieved as burst duration approached 500 μsec . The results are shown in Figure 7 wherein burst width 701 in μsec is plotted on the x-axis and Myosin Phosphorylation 702 as treated/sham is plotted on the y-axis. It can be seen that the PMF effect on Ca^{2+} binding to CaM approaches its maximum at approximately 500 μsec , just as illustrated by the Power SNR model.

These results confirm that a PMF signal, configured according to an embodiment of the present invention, would maximally increase myosin phosphorylation for burst durations sufficient to achieve optimal Power SNR for a given magnetic field amplitude.

Example 2

According to an embodiment of the present invention use of a Power SNR model was further verified in an *in vivo* wound repair model. A rat wound model has been well characterized both biomechanically and biochemically, and was used in this study. Healthy, young adult male Sprague Dawley rats weighing more than 300 grams were utilized.

The animals were anesthetized with an intraperitoneal dose of Ketamine 75 mg/kg and Medetomidine 0.5 mg/kg. After adequate anesthesia had been achieved, the dorsum was shaved, prepped with a dilute betadine/alcohol solution, and draped using sterile technique. Using a #10 scalpel, an 8-cm linear incision was performed through the skin down to the fascia on the dorsum of each rat. The wound edges were bluntly dissected to break any remaining dermal fibers, leaving an open wound approximately 4 cm in diameter. Hemostasis was obtained with applied pressure to avoid any damage to the skin edges. The skin edges were then closed with a 4-0 Ethilon running suture. Post-operatively, the animals received Buprenorphine 0.1-0.5mg/kg, intraperitoneal. They were placed in individual cages and received food and water *ad libitum*.

PMF exposure comprised two pulsed radio frequency waveforms. The first was a standard clinical PRF signal comprising a 65 μ sec burst of 27.12 MHz sinusoidal waves at 1 Gauss amplitude and repeating at 600 bursts/sec. The second was a PRF signal reconfigured according to an embodiment of the present invention. For this signal burst duration was increased to 2000 μ sec and the amplitude and repetition rate were reduced to 0.2G and 5 bursts/sec respectively. PRF was applied for 30 minutes twice daily.

Tensile strength was performed immediately after wound excision. Two 1 cm width strips of skin were transected perpendicular to the scar from each sample and used to measure the tensile strength in kg/mm². The strips were excised from the same

area in each rat to assure consistency of measurement. The strips were then mounted on a tensiometer. The strips were loaded at 10 mm/min and the maximum force generated before the wound pulled apart was recorded. The final tensile strength for comparison was determined by taking the average of the maximum load in kilograms per mm² of the two strips from the same wound.

The results showed average tensile strength for the 65 μ sec 1 Gauss PRF signal was 19.3 ± 4.3 kg/mm² for the exposed group versus 13.0 ± 3.5 kg/mm² for the control group ($p < .01$), which is a 48% increase. In contrast, the average tensile strength for the 2000 μ sec 0.2 Gauss PRF signal, configured according to an embodiment of the present invention using a Power SNR model was 21.2 ± 5.6 kg/mm² for the treated group versus 13.7 ± 4.1 kg/mm² ($p < .01$) for the control group, which is a 54% increase. The results for the two signals were not significantly different from each other.

These results demonstrate that an embodiment of the present invention allowed a new PRF signal to be configured that could be produced with significantly lower power. The PRF signal configured according to an embodiment of the present invention, accelerated wound repair in the rat model in a low power manner versus that for a clinical PRF signal which accelerated wound repair but required more than two orders of magnitude more power to produce.

Example 3

In this example Jurkat cells react to PMF stimulation of a T-cell receptor with cell cycle arrest and thus behave like normal T-lymphocytes stimulated by antigens at the T-cell receptor such as anti-CD3. For example in bone healing, results have shown both 60 Hz and PEMF fields decrease DNA synthesis of Jurkat cells, as is expected since PMF interacts with the T-cell receptor in the absence of a costimulatory signal. This is consistent with an anti-inflammatory response, as has been observed in clinical applications of PMF stimuli. The PEMF signal is more effective. A dosimetry analysis performed

according to an embodiment of the present invention demonstrates why both signals are effective and why PEMF signals have a greater effect than 60 Hz signals on Jurkat cells in the most EMF-sensitive growth stage.

5 Comparison of dosimetry from the two signals employed involves evaluation of the ratio of the Power spectrum of the thermal noise voltage that is Power SNR, to that of the induced voltage at the EMF-sensitive target pathway structure. The target pathway structure used is ion binding at receptor sites
10 on Jurkat cells suspended in 2 mm of culture medium. The average peak electric field at the binding site from a PEMF signal comprising 5msec burst of 200 μ sec pulses repeating at 15/sec, was 1mV/cm, while for a 60 Hz signal it was 50 μ V/cm.

Example 4

15 In this example electromagnetic field energy was used to stimulate neovascularization in an in vivo model. Two different signal were employed, one configured according to prior art and a second configured according to an embodiment of the present invention.

20 One hundred and eight Sprague-Dawley male rats weighing approximately 300 grams each, were equally divided into nine groups. All animals were anesthetized with a mixture of ketamine/acepromazine/Stadol at 0.1 cc/g. Using sterile surgical techniques, each animal had a 12 cm to 14 cm segment of
25 tail artery harvested using microsurgical technique. The artery was flushed with 60 U/ml of heparinized saline to remove any blood or emboli.

 These tail vessels, with an average diameter of 0.4 mm to 0.5 mm, were then sutured to the transected proximal and distal
30 segments of the right femoral artery using two end-to-end anastomoses, creating a femoral arterial loop. The resulting loop was then placed in a subcutaneous pocket created over the animal's abdominal wall/groin musculature, and the groin
35 incision was closed with 4-0 Ethilon. Each animal was then randomly placed into one of nine groups: groups 1 to 3

(controls), these rats received no electromagnetic field treatments and were killed at 4, 8, and 12 weeks; groups 4 to 6, 30 min. treatments twice a day using 0.1 gauss electromagnetic fields for 4, 8, and 12 weeks (animals were killed at 4, 8, and 12 weeks, respectively); and groups 7 to 9, 30 min. treatments twice a day using 2.0 gauss electromagnetic fields for 4, 8, and 12 weeks (animals were killed at 4, 8, and 12 weeks, respectively).

Pulsed electromagnetic energy was applied to the treated groups using a device constructed according to an embodiment of the present invention. Animals in the experimental groups were treated for 30 minutes twice a day at either 0.1 gauss or 2.0 gauss, using short pulses (2 msec to 20 msec) 27.12 MHz. Animals were positioned on top of the applicator head and confined to ensure that treatment was properly applied. The rats were reanesthetized with ketamine/acepromazine/Stadol intraperitoneally and 100 U/kg of heparin intravenously. Using the previous groin incision, the femoral artery was identified and checked for patency. The femoral/tail artery loop was then isolated proximally and distally from the anastomoses sites, and the vessel was clamped off. Animals were then killed. The loop was injected with saline followed by 0.5 cc to 1.0 cc of colored latex through a 25-gauge cannula and clamped. The overlying abdominal skin was carefully resected, and the arterial loop was exposed. Neovascularization was quantified by measuring the surface area covered by new blood-vessel formation delineated by the intraluminal latex. All results were analyzed using the SPSS statistical analysis package.

The most noticeable difference in neovascularization between treated versus untreated rats occurred at week 4. At that time, no new vessel formation was found among controls, however, each of the treated groups had similar statistically significant evidence of neovascularization at 0 cm² versus 1.42 ± 0.80 cm² ($p < 0.001$). These areas appeared as a latex blush segmentally distributed along the sides of the

arterial loop. At 8 weeks, controls began to demonstrate neovascularization measured at $0.7 \pm 0.82 \text{ cm}^2$. Both treated groups at 8 weeks again had approximately equal statistically significant ($p < 0.001$) outcroppings of blood vessels of $3.57 \pm 1.82 \text{ cm}^2$ for the 0.1 gauss group and of $3.77 \pm 1.82 \text{ cm}^2$ for the 2.0 gauss group. At 12 weeks, animals in the control group displayed $1.75 \pm 0.95 \text{ cm}^2$ of neovascularization, whereas the 0.1 gauss group demonstrated $5.95 \pm 3.25 \text{ cm}^2$, and the 2.0 gauss group showed $6.20 \pm 3.95 \text{ cm}^2$ of arborizing vessels. Again, both treated groups displayed comparable statistically significant findings ($p < 0.001$) over controls.

These experimental findings demonstrate that electromagnetic field stimulation of an isolated arterial loop according to an embodiment of the present invention increases the amount of quantifiable neovascularization in an in vivo rat model. Increased angiogenesis was demonstrated in each of the treated groups at each of the sacrifice dates. No differences were found between the results of the two gauss levels tested as predicted by the teachings of the present invention.

Having described embodiments for an apparatus and a method for delivering electromagnetic treatment to human, animal and plant molecules, cells, tissue and organs, it is noted that modifications and variations can be made by persons skilled in the art in light of the above teachings. It is therefore to be understood that changes may be made in the particular embodiments of the invention disclosed which are within the scope and spirit of the invention as defined by the appended claims.

What is claimed is:

1) A method for electromagnetic treatment of living tissues and cells by enhancing angiogenesis and neovascularization comprising the steps of:

Configuring at least one waveform according to a mathematical model having at least one waveform parameter, said at least one waveform to be coupled to a angiogenesis and neovascularization target pathway structure;

Choosing a value of said at least one waveform parameter so that said at least waveform is configured to be detectable in said angiogenesis and neovascularization target pathway structure above background activity in said angiogenesis and neovascularization target pathway structure;

Generating an electromagnetic signal from said configured at least one waveform; and

Coupling said electromagnetic signal to said angiogenesis and neovascularization target pathway structure using a coupling device.

2) The method of claim 1, wherein said at least one waveform parameter includes at least one of a frequency component parameter that configures said at least one waveform to repeat between about 0.01 Hz and about 100 MHz, a burst amplitude envelope parameter that follows a mathematically defined amplitude function, a burst width parameter that varies at each repetition according to a mathematically defined width function, a peak induced electric field parameter varying between about 1 $\mu\text{V}/\text{cm}$ and about 100 mV/cm in said target pathway structure according to a mathematically defined function, and a peak induced magnetic electric field parameter varying between about 1 μT and about 0.1 T in said target pathway structure according to a mathematically defined function.

3) The method of claim 1, wherein said angiogenesis and neovascularization target pathway structure includes at least one of ions and ligands.

5 4) The method of claim 1, further comprising the step of binding ions and ligands to regulatory molecules in living cells and tissues thereby modulating angiogenesis and neovascularization.

10 5) The method of claim 4, wherein said binding of ions and ligands includes modulating Calcium to Calmodulin binding.

15 6) The method of claim 4, wherein said binding of ions and ligands includes modulating growth factor production in living cells and tissues.

7) The method of claim 4, wherein said binding of ions and ligands includes modulating cytokine production in living cells and tissues.

20 8) The method of claim 4, wherein said binding of ions and ligands includes modulating growth factors and cytokines relevant to angiogenesis and neovascularization.

25 9) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of bone fractures and disorders.

30 10) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of cardiovascular diseases.

35 11) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of cerebral diseases.

12) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of cerebrovascular disease.

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13) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment peripheral vascular disease.

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14) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of diseased or ischemic cells and tissues.

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15) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of an acute or chronic soft tissue wound.

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16) The method of claim 4, wherein said binding of ions and ligands includes modulating angiogenesis and neovascularization for treatment of sprains strains and contusions.

17) An electromagnetic treatment apparatus for plants, animals, and humans to enhance angiogenesis and neovascularization comprising:

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A waveform configuration means for configuring at least one waveform to be coupled to a angiogenesis and neovascularization target pathway structure according to a mathematical model having at least one waveform parameter capable of being chosen so that said at least one waveform is configured to be detectable in said angiogenesis and neovascularization target structure above background activity in said angiogenesis and neovascularization target pathway structure;

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An electromagnetic signal generating means connected to said waveform device by at least one connecting means for

generating an electromagnetic signal from said configured at least one waveform; and

A coupling device connected by at least one connecting means to said electromagnetic signal generating device for coupling said electromagnetic signal to said angiogenesis and neovascularization target pathway structure.

18) The electromagnetic treatment apparatus of claim 17, wherein said at least one waveform parameter includes at least one of a frequency component parameter that configures said at least one waveform to repeat between about 0.01 Hz and about 100 MHz according to a mathematical function, a burst amplitude envelope parameter that follows a mathematically defined amplitude function, a burst width parameter that varies at each repetition according to a mathematically defined width function, a peak induced electric field parameter varying between about 1 $\mu\text{V}/\text{cm}$ and about 100 mV/cm in said angiogenesis and neovascularization target pathway structure according to a mathematically defined function, and a peak induced magnetic electric field parameter varying between about 1 μT and about 0.1 T in said angiogenesis and neovascularization target pathway structure according to a mathematically defined function.

19) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues wherein Calcium binding to Calmodulin is modulated.

20) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues wherein Calcium binding to Calmodulin is modulated.

21) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and

tissues wherein growth factors and cytokines relevant to angiogenesis and neovascularization are modulated.

22) The electromagnetic signal generating means of claim 21
5 wherein the growth factors include at least one of fibroblast growth factors, platelet derived growth factors and interleukin growth factors.

23) The electromagnetic signal generating means of claim 17
10 wherein the signal is capacitively coupled to living cells and tissues wherein growth factors and cytokines relevant to angiogenesis and neovascularization are modulated.

24) The electromagnetic signal generating means of claim 23
15 wherein the growth factors include at least one of fibroblast growth factors, platelet derived growth factors and interleukin growth factors.

25) The electromagnetic signal generating means of claim 17
20 wherein the signal is inductively coupled to living cells and tissues to modulate growth factor production.

26) The electromagnetic signal generating means of claim 17
25 wherein the signal is capacitively coupled to living cells and tissues to modulate growth factor production.

27) The electromagnetic signal generating means of claim 17
wherein the signal is inductively coupled to living cells and tissues to modulate cytokine production.

28) The electromagnetic signal generating means of claim 17
30 wherein the signal is capacitively coupled to living cells and tissues to modulate cytokine production.

29) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of bone fractures and disorders.

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30) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of bone fractures and disorders.

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31) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of cardiovascular diseases.

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32) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of cardiovascular diseases.

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33) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of cerebral diseases.

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34) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of cerebral diseases.

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35) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of cerebrovascular disease.

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36) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of cerebrovascular disease.

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37) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of peripheral vascular disease.

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38) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of peripheral vascular disease.

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39) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of diseased or ischemic cells and tissues.

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40) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of diseased or ischemic cells and tissues.

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41) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of an acute or chronic soft tissue wound.

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42) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of an acute or chronic soft tissue wound.

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43) The electromagnetic signal generating means of claim 17 wherein the signal is inductively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of sprains strains and contusions.

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44) The electromagnetic signal generating means of claim 17 wherein the signal is capacitively coupled to living cells and tissues to modulate angiogenesis and neovascularization for the treatment of sprains strains and contusions.

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