# EMF as First Messengers in Biological Signaling

## Part I: A General Mechanism for Non-Thermal Bioeffects

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**ABSTRACT**

This study presents a model and supporting experimental evidence that non-thermal EMF can act as first messengers in calmodulin (CaM)-dependent signaling pathways. The second messenger is free cytosolic $Ca^{2+}$, for which CaM is a primary responder when homeostasis is disrupted. Transient elevations in $Ca^{2+}$ rapidly activate CaM via voltage-dependent $Ca^{2+}$ binding, which is kinetically asymmetrical, i.e., binding is significantly faster than release ($k_{on} >> k_{off}$). Such asymmetry gives Ca/CaM binding rectifier-like properties, suggesting that any bipolar PEMF signal for which pulse duration or carrier period is significantly lower than bound lifetime will modulate Ca/CaM binding. Activated CaM initiates nitric oxide (NO) signaling cascades relevant to tissue growth, repair and maintenance. *In vitro* studies using antagonists and inhibitors provide strong support that PEMF, configured with frequency content of sufficient amplitude within the bandpass of Ca/CaM binding kinetics to be detectable above thermal noise, will modulate CaM-dependent NO signaling. Randomized, double-blind clinical studies provide additional support for the proposed PEMF mechanism. Because $Ca^{2+}$ binding is voltage-dependent with forward reaction favored, such PEMF signals will also modulate CaM-dependent heat shock protein (HSP) release through NO signaling in quiescent cells, suggesting that PEMF can have protective bioeffects. Taken together, results suggest that PEMF signals configured according to the proposed mechanism will have significant efficacy for a wide range of clinical and wellness applications.

## INTRODUCTION

In 1972, the Electrochemical Information Transfer (ECM) model [Pilla, 1972; 1974a; 1974b; 1976; 2006] proposed that weak non-thermal electromagnetic fields (EMF) could be configured to modulate voltage-dependent (electrochemical) processes at electrified aqueous cell interfaces. The model ascribed a signaling function to exogenous non-thermal electric fields by modulating ion binding and/or transmembrane transport in such a way as to affect pathways mediated by second messengers. This guided the *a priori* configuration of bone growth stimulator (BGS) signals that are now part of the standard armamentarium of orthopedic practice worldwide for the treatment of recalcitrant bone fractures [Bassett et al., 1977; Mammi et al., 1993 ; Nelson et al., 2003; Aaron et al., 2004; 2006 ]. In separate studies, radio frequency signals, originally developed for deep tissue heating (diathermy), were shown to produce biological effects when applied at non-thermal levels using pulse-modulation techniques to produce pulsed radio frequency (PRF) signals [Salzberg et al., 1995; Kloth et al., 1999; Pilla et al., 1996; Pennington et al., 1993; Foley-Nolan et al., 1990; 1992; Mayrovitz and Larsen, 1992; 1995; Mayrovitz et al., 2002; Porreca et al., 2008; Frykberg et al., 2009; Strauch et al., 2009]. At the cellular level, numerous studies demonstrate that BGS, PRF and other EMF signals modulate the release of growth factors and cytokines [Seegers et al., 2001; Brighton et al., 2001; Aaron et al., 2004; Tepper et al., 2004; Li et al., 2007; Callaghan et al., 2008; Vianale et al., 2008; Fitzsimmons et al., 2008; Rohde et al., 2009], some of which were associated with the biological effects of the same signals.

Based on all of the above, as well as the findings that electromagnetic fields modulate calmodulin (CaM)-dependent enzyme activity [Markov et al., 1993; 1994; Markov and Pilla, 1997; Engstrom et al., 2002; Liboff et al., 2003], bioeffective PRF signals were configured *a priori* using the ECM model along with evaluation of the signal to thermal noise ratio (SNR) for this target pathway [Pilla et al., 1994; 1999]. This led to the suggestion that a non-thermal EMF signal could be configured to act as a first messenger capable of modulating physiologically meaningful CaM-dependent signaling pathways [Pilla, 2007] that are normally activated by transient changes in cytosolic concentrations of free calcium ions ($Ca^{2+}$) [Berridge et al., 2003]. One such pathway is the CaM-dependent nitric oxide (NO)/guanylyl cyclase (sGC) signaling pathway, which rapidly modulates the response to departures from intracellular $Ca^{2+}$ homeostasis in various tissues. This cascade is involved in

the production of heat shock proteins (HSP) [Kim et al., 1997; Steensberg et al., 2007; Li et al., 2008; Manucha and Vallés, 2008], which play important roles in the inflammatory, repair and regenerative phases of healing, and tissue maintenance [Madhusoodanan and, Murad, 2007]. Here, it is shown how EMF signals may be configured *a priori* to modulate a two-step ion binding pathway involving $Ca^{2+}$ binding to calmodulin (CaM), followed by CaM activation of constitutive nitric oxide synthases (cNOS), resulting in the catalytic synthesis of NO. The importance of CaM-dependent NO signaling in tissue growth, repair and maintenance is discussed and supporting evidence that non-thermal EMF signals can affect this pathway is provided at the molecular and cellular levels as well as in animal models and clinical studies with human subjects.

## THE BIOLOGICAL SIGNALING PATHWAY

The biological pathway proposed to be sensitive to non-thermal EMF is the voltage-dependent binding of $Ca^{2+}$ to CaM. Basal levels of intracellular $Ca^{2+}$ are typically 50–100 nM, tightly maintained by a number of physiological calcium buffers [Weissman et al., 2002]. Transient elevations in cytosolic $Ca^{2+}$ from external stimuli as simple as changes in temperature and mechanical forces, or as complex as mechanical disruption of tissue, rapidly activates CaM [Colomer and Means, 2007] which equally rapidly activates the cNOS enzymes, i.e., endothelial and neuronal NOS, or eNOS and nNOS, respectively. Studies have shown that both forms are inactive at basal intracellular levels of $Ca^{2+}$, however, their activity increases with elevated $Ca^{2+}$, reaching half-maximal activity at about 300 nM [Bredt and Snyder, 1990; Schmidt et al., 1991]. Thus, nNOS and eNOS are regulated by changes in intracellular $Ca^{2+}$ concentrations within the physiological range. In contrast, a third, inducible isoform of NOS (iNOS), which is upregulated during inflammation by macrophages and/or neutrophils [Moilanen and Vapaatalo, 1995], contains CaM that is tightly bound, even at low resting levels of cytosolic $Ca^{2+}$ [Cho et al., 1992], and is not sensitive to intracellular $Ca^{2+}$.

Once cNOS is activated by CaM it converts its substrate, L-arginine, to citrulline, releasing one molecule of NO [Knowles et al., 1994]. As a gaseous free radical with a half-life of about 5 sec [Ignarro et al., 1993], NO diffuses locally through membranes and organelles and acts on molecular targets at a distance up to 200 μm [Malinski et al., 1993; Tsoukias, 2008]. The low transient concentrations of NO from cNOS can activate soluble guanylyl cyclase (sGC), which catalyzes the synthesis of cyclic guanosine monophosphate (cGMP) [Cho et al., 1992]. The CaM/NO/cGMP signaling pathway is a rapid response cascade which can

modulate peripheral and cardiac blood flow in response to normal physiologic demands, as well as to inflammation [Bredt, 2003]. This same pathway also modulates the release of cytokines, such as interleukin-1beta (IL-1β) [Ren and Torres, 2009; Naldini and Carraro, 2005] and growth factors such as basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF) [Madhusoodanan and, Murad, 2007; Werner and Grose, 2003] which have pleiotropic effects on cells involved in tissue repair and maintenance.

Following an injury, e.g., a bone fracture or a surgical incision, repair commences with an inflammatory stage during which the pro-inflammatory cytokine IL-1β is rapidly released. This, in turn, up-regulates iNOS, resulting in the production of large amounts of NO in the wound bed [LaPointe and Isenović, 1999; Lee et al., 2001]. Continued exposure to NO leads to the induction of cyclooxygenase-2 and increased synthesis of prostaglandins which also play a role in the inflammatory phase [Lee et al., 2001]. While this process is a natural component of healing, when it is protracted it can lead to increased pain and delayed or abnormal healing [Broughton et al., 2006]. In contrast, CaM/eNOS/NO signaling has been shown to attenuate levels of IL-1β and down-regulate iNOS [Palmi and Meini, 2002; Zhao et al., 2007]. Non-thermal PEMF has been reported to down-regulate iNOS at the mRNA and protein levels in monocytes [Reale et al., 2006], and pro-inflammatory cytokines in human keratinocytes [Vianale et al., 2008]. PEMF also modulates the sequential expression of iNOS, eNOS and cyclooxygenase-2 (COX-2) in human keratinocytes, although in this study this is almost certainly a magnetic field effect [Patruno et al., 2010]. Finally, weak electric fields partially reversed the decrease in the production of extracellular matrix caused by exogenous IL-1β in full-thickness articular cartilage explants from osteoarthritic adult human knee joints [Brighton et al., 2008]. All of these results provide strong support for the frequently reported anti-inflammatory effects of PEMF at early stages of tissue repair.

As tissue further responds to injury, the CaM/NO/cGMP cascade is activated in endothelial cells to stimulate angiogenesis, without which new tissue growth cannot be sustained. Evidence that non-thermal EMF can modulate this cascade is provided by several studies. An early study showed that the original BGS signal promoted the creation of tubular, vessel-like, structures from endothelial cells in culture in the presence of growth factors [Yen-Patton et al., 1998]. Another study using the same BGS signal confirmed a seven-fold increase in endothelial cell tubularization in vitro [Tepper et al., 2004]. Quantification of angiogenic

proteins demonstrated a five-fold increase in FGF-2, suggesting that PEMF stimulates angiogenesis by increasing FGF-2 production. This same study also reported PEMF increased vascular in-growth more than two-fold when applied to an implanted Matrigel plug in mice, with a concomitant increase in FGF-2, similar to that observed in vitro. The BGS signal significantly increased neovascularization and wound repair in normal mice, and particularly in diabetic mice, through an endogenous increase in FGF-2, which could be eliminated by using a FGF-2 inhibitor [Callaghan et al., 2008]. Similarly, a pulse modulated radio frequency (PRF) signal of the type used clinically for wound repair was reported to significantly accelerate vascular sprouting from an arterial loop transferred from the hindlimb to the groin in a rat model [Roland et al., 2000]. This study was extended to examine free flap survival on the newly produced vascular bed [Weber et al., 2005]. Results showed 95% survival of PRF-treated flaps compared to 11% survival in the sham-treated flaps, suggesting a significant clinical application for PRF signals in reconstructive surgery**.** Finally, a recent study [Delle Monache et al., 2008] showed that a 1 mT 50 Hz signal increased the degree of endothelial cell proliferation and tubule formation and accelerated the process of wound repair, suggesting a mechanism based upon a PEMF effect on VEGF receptors.

Non-thermal EMF applied before an injury can protect or accelerate healing from ischemia-reperfusion and other insults without relying on potentially compromised blood circulation to carry a pharmacological substance to the injury site [Albertini et al., 1999; Alfieri et al., 2006; Grant et al., 1994; DiCarlo et al., 1999; Ronchi et al., 2004; Robertson et al., 2007; George et al., 2008]. Three of these studies showed that tissue protection was related to an increase, by EMF, in the release of HSP70, a molecular chaperone that increases cell survival, before the injury [DiCarlo et al., 1999; Ronchi et al., 2004; George et al., 2008]. It should be noted that NO signaling can modulate HSP release, suggesting that these effects were mediated by the up-stream induction of NO by EMF.



**PROPOSED NON-THERMAL EMF TRANSDUCTION PATHWAY**

The proposed EMF transduction pathway relevant to tissue maintenance, repair and regeneration, begins with voltage-dependent $Ca^{2+}$ binding to CaM followed by Ca/CaM binding to and subsequent activation of cNOS, which catalyzes the synthesis of the signaling molecule NO from L-arginine. This pathway is shown in its simplest schematic form in Figure 1.

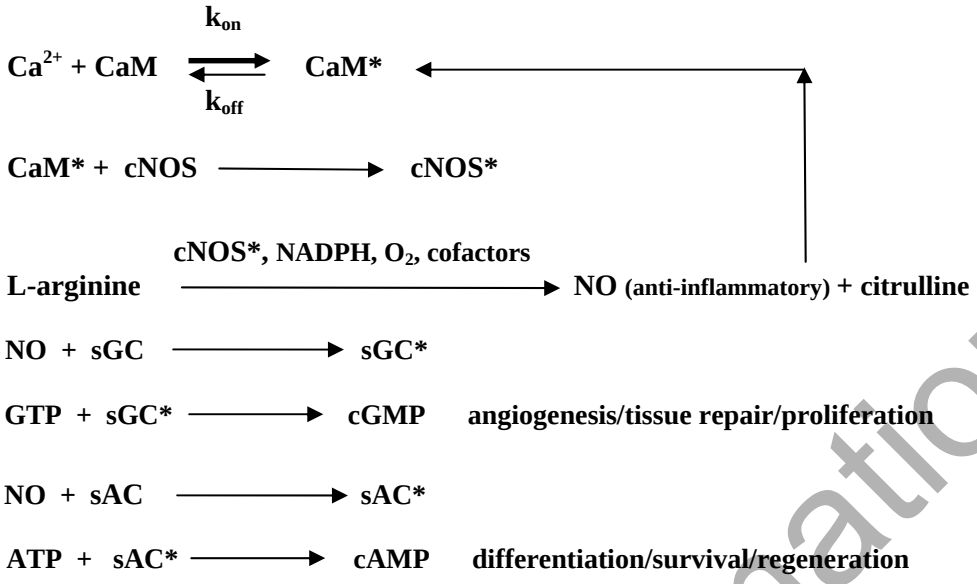


**Figure 1.** Schematic representation of the proposed EMF transduction pathway. Here EMF modulates $Ca^{2+}$ binding to CaM, wherein $k_{on}$ is voltage-dependent and $k_{on} >> k_{off}$, imparting rectifier-like properties to $Ca^{2+}$ binding. cNOS* represents activated cNOS (typically a homodimer[73]), sGC* is activated guanylyl cyclase when NO signaling modulates the tissue repair pathway; sAC* is activated adenylyl cyclase when NO signaling modulates differentiation and survival.



As may be seen, Ca/CaM binding is kinetically asymmetrical, i.e., the rate of binding exceeds the rate of dissociation by several orders of magnitude ($k_{on} >> k_{off}$), driving the reaction in the forward direction. Ca/CaM binding has been well characterized, with the binding time constant reported to be in the range of $10^{-2}$-$10^{-3}$ sec [Blumenthal and Stull, 1982]. In contrast, release of $Ca^{2+}$ from CaM cannot occur until cNOS* has converted L-arginine to citrulline and NO, which takes the better part of a second [Daff, 2003]. Subsequent reactions involving NO depend upon the cell/tissue state. For example, tissue repair requires a temporal sequence of inflammatory, anti-inflammatory, angiogenic and proliferative components. Endothelial cells orchestrate the production of FGF-2 and VEGF for angiogenesis [Werner and Grose, 2003]. For each of these phases, early NO production by endothelial cells, leading to increased cGMP by these, as well as other NO targets, such as vascular smooth muscle, would be expected to be modulated by a PEMF effect on CaM. In contrast, nerve or bone regeneration may require other pathways leading to differentiation during development and growth and prevention of apoptosis, as in response to injury or neurodegenerative diseases. For these cases, the prediction is that early cAMP formation would be modulated by a PEMF effect on CaM.

The substantial asymmetry of Ca/CaM binding kinetics provides a unique opportunity to configure EMF signals that selectively modulate $k_{on}$. In general, if $k_{on} >> k_{off}$, and $k_{on}$ is voltage-dependent, binding could be increased with any bipolar signal having a carrier period or pulse duration that is significantly lower than the mean lifetime of the bound ion. This applies to the CaM signaling pathway, causing it to exhibit rectifier-like properties, i.e., to yield a net increase in the population of bound $Ca^{2+}$ in response to any bipolar waveform with pulse duration or carrier period significantly lower than the bound lifetime. What follows will demonstrate how a non-thermal PEMF signal can be configured to optimally modulate $Ca^{2+}$ binding to CaM using the ECM model by assessing its electrical detectability in the binding pathway.

The change in surface concentration, $\Delta\Gamma$, of $Ca^{2+}$ at CaM is equal to the net increase in the number of ions that exit the outer Helmholtz plane, penetrate the water dipole layer at the aqueous interface of the binding site, and become bound in the inner Helmoltz plane [Pilla, 1976]. As shown for the general case of ion binding elsewhere [Pilla et al., 1994; 1999], evaluation of Ca/CaM binding impedance, $Z_A(s)$, will allow calculation of the detectability of any given waveform in that pathway by calculating the signal-to-(thermal) noise ratio (SNR). Thus, binding current, $I_A(t)$, is proportional to the change in surface charge (bound ion concentration) via dq(t)/dt, or, in the frequency domain, via $sq_A(s)$. $I_A(s)$ is, thus, given by:

$$I_A(s) = s\, q_A(s) = s\,\Gamma_o\, f\left(\Delta\Gamma(s)\right) \qquad \textbf{(1)}$$

where s is the real-valued frequency variable of the LaPlace transform [Cheng, 1970; Pilla, 1970]. Taking the first term of the Taylor expansion of equation 1 gives:

$$I_A(s) = q_\Gamma\, s\,\Gamma_o\,\Delta\Gamma(s) \qquad \textbf{(2)}$$

where $q_\Gamma = \partial q/\partial\Gamma$, a coefficient representing the dependence of surface charge on bound ion concentration. $\Delta\Gamma(s)$ is a function of the applied voltage waveform, E(s), and, referring to the reaction scheme in figure 1, of the change in concentration of eNOS*, defined as $\Delta\Phi(s)$:

$$\Delta\Gamma(s) = k_{on}/\Gamma_o s\,[-\Delta\Gamma(s) + a\,E(s) + \Delta\Phi(s)] \qquad \textbf{(3)}$$

where $\Gamma_o$ is the initial surface concentration of $Ca^{2+}$ (homeostasis), and $a = \partial\Gamma/\partial E$, representing the voltage dependence of $Ca^{2+}$ binding. Referring to the reaction scheme in figure 1, it may also be seen that eNOS* depends only upon $Ca^{2+}$ binding, i.e., $\Delta\Gamma(s)$. Thus:

$$\Delta\Phi(s) = \upsilon_\Phi/\Phi_o s\,[-\Delta\Phi(s) - \Delta\Gamma(s)] \qquad \textbf{(4)}$$

where $\upsilon_\Phi$ is the rate constant for CaCaM binding to eNOS and $\Phi_o$ is the initial concentration of eNOS* (homeostasis).

Using equations 2, 3 and 4, and for $k_{on} >> \upsilon_\Phi$, $Z_A(s)$ may be written:

$$Z_A(s) = \frac{E(s)}{I_A(s)} = \frac{1}{q_\Gamma a}\left[\frac{1+\Gamma_o s/k_{on}}{\Gamma_o s}\right] \qquad \textbf{(5)}$$

Inspection of equation 5 reveals that the $Ca^{2+}$ binding impedance is functionally equivalent to that of a series $R_A - C_A$ equivalent electric circuit (see Figure 2), which forms part of the overall membrane impedance [Pilla, 1974; 2006]. Here, $R_A$ and $C_A$ are the equivalent resistance and capacitance of binding kinetics, respectively. For this circuit, the impedance can be written as $Z = (1 + sR_AC_A)/sC_A$, where $C_A$, is:

$$C_A = q_\Gamma a\,\Gamma_o \qquad \textbf{(6)}$$

which shows that $C_A$ is directly proportional to bound $Ca^{2+}$, and $R_A$ is:

$$R_A = 1/(q_\Gamma a\, k_{on}) \qquad \textbf{(7)}$$

indicating that $R_A$ is inversely proportional to the $Ca^{2+}$ binding rate constant $k_{on}$. Note that $q_\Gamma a = (\partial q/\partial\Gamma)\cdot(\partial\Gamma/\partial E) = \partial q/\partial E$, a proportionality constant relating to the voltage dependence of surface charge for the binding ion.

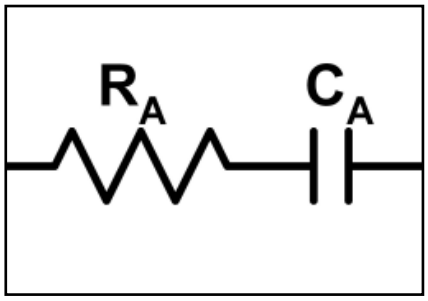


**Figure 2:** Equivalent electric circuit representing asymmetrical binding impedance from equation 5, as occurs for $Ca^{2+}$ binding to CaM. The binding time constant is $\tau_A = R_A C_A$, in which $R_A$ is inversely proportional to $k_{on}$ and $C_A$ is directly proportional to bound $Ca^{2+}$ ($\Delta\Gamma$).



## SIGNAL TO THERMAL NOISE RATIO

The signal to (thermal) noise ratio, SNR, is a primary index of the detectability of a given PEMF signal to increase bound $Ca^{2+}$, i.e., a measure of the ability of a PEMF signal to modulate $Ca^{2+}$ binding in the target pathway. In other words SNR is a measure of signal detection (above thermal noise voltage) in the target pathway. To evaluate SNR for the Ca/CaM target, the quantity of interest is the effective voltage, $E_b(s)$, induced across the equivalent binding capacitance, $C_A$ (see figure 2), which is directly proportional to $Ca^{2+}$ as defined in equation 6, and given by [Cheng, 1970]:

$$E_b(s) = \frac{(1/sC_A)\,E(s)}{(R_A^2 + (1/sC_A)^2)^{1/2}} \qquad \textbf{(8)}$$

where $R_A$ is the equivalent binding resistance (see equation 7). Equation 8 describes the relation between the frequency response of binding, $E_b(s)$, and applied field waveform E(s), clearly illustrating that EMF response is dependent upon applied waveform parameters.

Thermal noise in the binding pathway can be evaluated via [DeFelice, 1981]:

$$RMS_{noise} = \left[ 4kT \int_{\omega_1}^{\omega_2} \mathrm{Re}[Z_A(x,\omega)\,d\omega \right]^{1/2} \qquad \textbf{(9)}$$

where Re is the real part of the total binding impedance, $Z_A$, and the limits of integration ($\omega_1$, $\omega_2$) are determined by the bandpass of binding, typically $10^{-2}$-$10^{7}$ rad/sec. SNR is evaluated using $E_b(s)/RMS_{noise}$, however, the binding time constant $\tau_A=R_AC_A$, must be known. In the case of $Ca^{2+}$ binding to CaM, $\tau_A$ has been reported to be in the range of $10^{-2}$-$10^{-3}$ sec [Blumenthal and Stull, 1982]. Peak SNR should therefore occur between s = $10^2$ and $10^3$ rad/sec for any applied waveform. $E_b(s)$ is evaluated by taking the Laplace transform of the input signal E(s), which is positive-valued at all frequencies and accounts for Ca/CaM binding asymmetry. An example of an SNR calculation which predicts the effect of burst duration for a pulse modulated 27.12 MHz radio frequency waveform (PRF) on Ca/CaM binding is shown in Figure 3. Here it may be seen that for a non-thermal amplitude of 50 mG, a burst duration of 2 msec is required to produce sufficient SNR to modulate $Ca^{2+}$ binding in CaM-dependent signaling pathways. As expected, peak SNR falls within the frequency range defined by $\tau_A$, within which the 2 msec burst waveform has more amplitude compared to that for the 0.1 msec burst waveform.

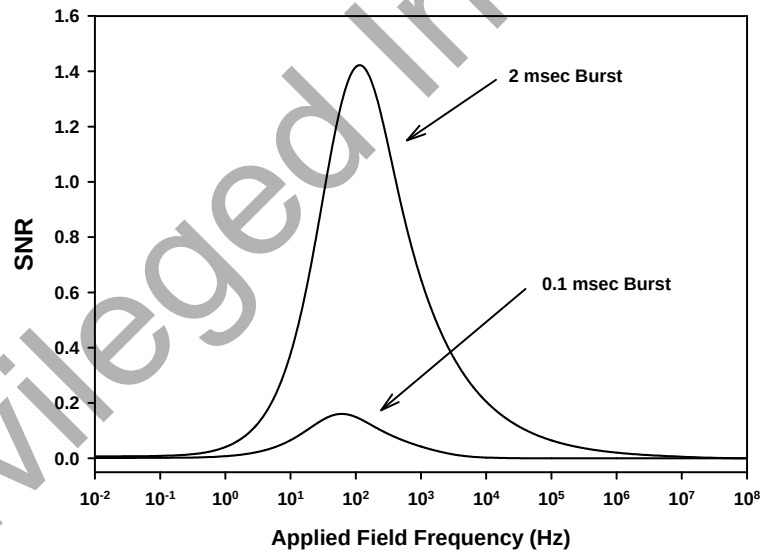


**Figure 3:** SNR analysis illustrating the effect of burst duration in a Ca/CaM pathway for a pulse modulated 27.12 MHz waveform repeating at 1 Hz with 50 mG peak amplitude. Only the 2 msec burst produced sufficient SNR at 50 mG in the frequency range defined by $\tau_A$ for detectability in the Ca/CaM pathway.

## EXPERIMENTAL EVIDENCE SUPPORTING THE PROPOSED EMF TRANSDUCTION MECHANISM

### Cell-Free CaM-Dependent Enzyme Assay

Early applications of the ECM/SNR model were carried out using a cell-free enzyme assay for CaM-dependent myosin light chain kinase [Markov et al., 1994; Markov and Pilla, 1997]. Those studies confirmed the predicted dependence of myosin phosphorylation on free $Ca^{2+}$ and were the first to show a dosimetric dependence of a CaM-dependent enzyme on the on the burst duration of a pulse modulated 27.12 MHz radio frequency signal (PRF) applied at 50 mG, for which the mean induced electric field was 21 V/m [Pilla et al., 1994; Pilla, 2006]. Results are summarized in Figure 4, demonstrating that only burst durations of approximately 1 msec and above produced a significant increase in myosin phosphorylation, in accord with predictions from the ECM/SNR model, which assumes that the PRF signal modulates $Ca^{2+}$ binding to CaM. The PEMF effect appears to level off at approximately 4 msec, which is well within the frequency range defined by $\tau_A$ for $Ca^{2+}$ binding to CaM.

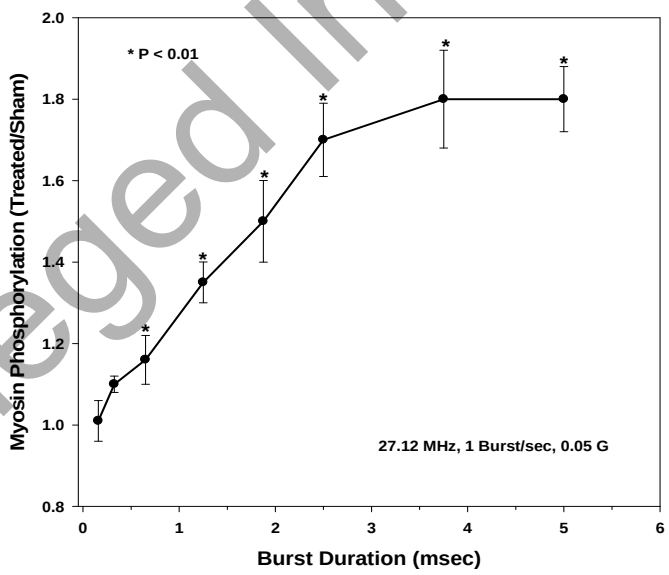


**Figure 4:** Effect of burst duration of a pulse modulated 27.12 MHz carrier on CaM-dependent myosin phosphorylation in an enzyme assay. These results show only burst durations of approximately 1 msec and above produced a significant increase in phosphorylation, in accord with the ECM/SNR model. At 50 mG, burst durations above about 4 msec produce no additional PEMF effect.

**Articular Chondrocytes**

The BGS signal, as employed clinically, yields an SNR that is 3-fold lower for the Ca/CaM target, but this signal, configured *a priori* with the ECM model, is still sufficient to produce physiologically significant bioeffects. For example, a single 30 min exposure of a capacitively coupled BGS signal increased DNA synthesis in articular chondrocytes by 150% over 72 hours. The conclusion that PEMF acted as a first messenger to modulate CaM-dependent NO/cGMP signaling was confirmed systematically using N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7), a CaM antagonist, to inhibit CaM activation of cNOS, L-nitrosoarginine methyl ester (L-NAME), a cNOS inhibitor, and 6-anilino-5,8-quinolinedione (LY83583) a sGC inhibitor. When these inhibitors were present during PEMF exposure, each could independently eliminate the PEMF effect on DNA synthesis at 72 hours [Fitzsimmons et al., 2008]. These results provide direct support for the proposal that PEMF acts as a first messenger to modulate CaM-dependent cascades, such as NO/cGMP signaling, which would in turn enhance tissue repair and cell proliferation [Pilla 2007]. Abolition of the PEMF effect on cGMP at 15 min by W-7 and L-NAME is shown in Figure 5A, and inhibition by L-NAME and LY83583 on DNA synthesis in Figure 5B.

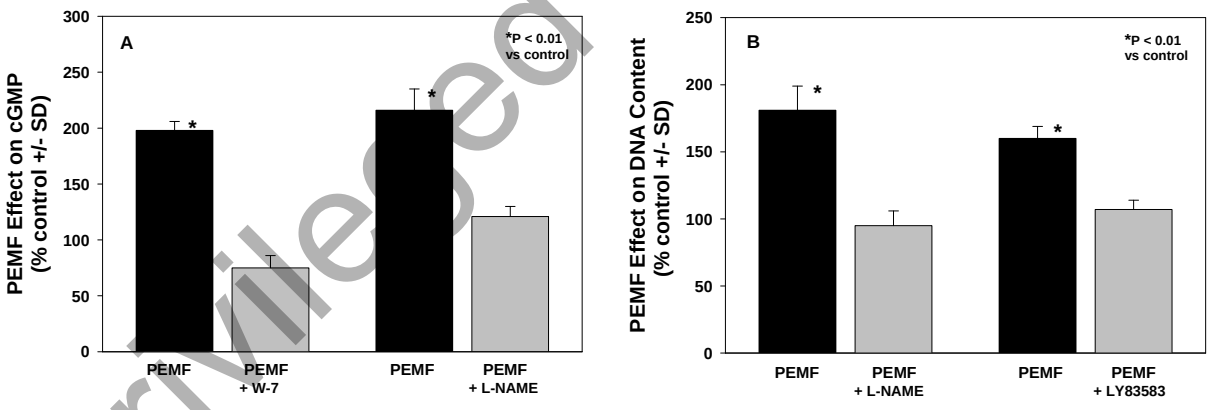


**Figure 5:** Effect of a single 30 min PEMF (BGS) exposure on human articular chondrocytes. PEMF increases short-term (15 min) cGMP generation which was inhibited by the CaM antagonist W-7 and, independently, by the cNOS inhibitor L-NAME (**A**). PEMF increased DNA synthesis at 72 hours, which was completely blocked at the signaling stage by the cNOS inhibitor L-NAME and by the sGC inhibitor LY83583, which blocked the PEMF increase in cGMP formation by more than 90% (**B**). These results provide strong direct evidence for the proposed PEMF transduction pathway. *From Fitzsimmons et al., 2008, with permission.*

**Endothelial Cells**

The proposed EMF transduction pathway (Figure 1) suggests that the effect of PEMF on the synthesis of NO from eNOS, by modulation of $Ca^{2+}$ binding to CaM, would potentially modulate angiogenesis [Broughton et al., 2006]. Indeed, angiogenesis requires the production of fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF), which first require that eNOS be activated to catalyze the formation of NO from endothelial cells [Fukumura et al., 2001; Cooke, 2003; Duda et al., 2004]. This makes the modulation of eNOS activity a useful strategy to modulate angiogenesis for tissue repair and possibly other conditions that require vascular plasticity, such as ischemia [Cooke, 2003]. Endothelial cells would therefore be a likely target for PEMF modulation of tissue repair. The expectation is that PEMF will modulate cGMP formation at the signaling level and increase FGF-2 to stimulate angiogenesis.

A recent study examined the effect of a PRF waveform configured to modulate the CaM/NO/cGMP pathway on cGMP production from human umbilical vascular endothelial cells (HUVECs) in culture [Wu et al., 2010]. The details of this study are presented in Part III of this series. A summary is presented here. HUVEC cells were removed from the incubator and exposed to a PRF signal that consisted of a 3 msec burst of 27.12 MHz sinusoidal waves repeating at 3 bursts/sec. The average induced electric field was 32 ± 6 mV/cm. Cultures were exposed at room temperature for 15 min to PRF within the central portion of a single-turn 20 cm diameter coil (antenna). Exposure at room temperature was a repeatable "injury" designed to increase intracellular $Ca^{2+}$ from its homeostatic levels. Cultures remained at room temperature for an additional 15 min after PRF exposure, after which cells were lysed for cGMP analysis. In some cultures trifluoroperazine (TFP) a CaM antagonist [Colomer et al., 2010] was employed to verify whether the activation of CaM by $Ca^{2+}$ binding was required by PEMF to increase levels of cGMP. Results demonstrate that a single 15-minute exposure to PRF produced nearly a 3-fold increase in cGMP, which was inhibited by TFP (Fig. 6). This suggests that a PRF signal configured to modulate the CaM/NO/cGMP signaling pathway would be able to increase or accelerate the angiogenesis required for tissue repair. These results provide mechanistic support for the reported acceleration of cutaneous wound repair by 59% and Achilles' tendon repair by 69% at 21 days in rat models using an identical PRF signal [Strauch et al.,2006a; 2007]. The results also provide a mechanistic explanation for the observed effects of the BGS signal on HUVEC tubule formation and wound repair, both of which involve FGF-2 [Tepper et al., 2004; Callaghan et al., 2008].

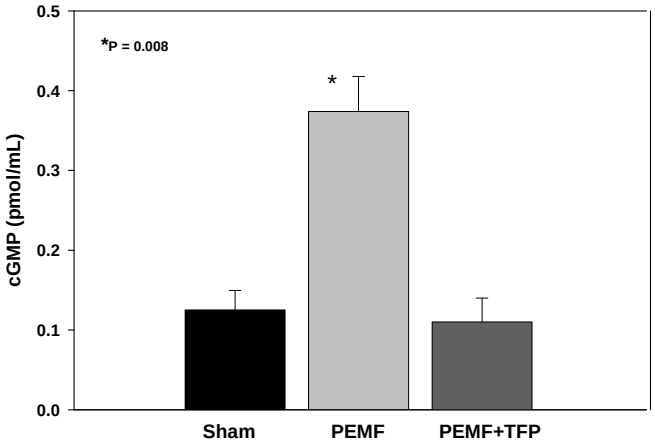


**Figure 6:** Effect of PRF configured to modulate CaM/NO/cGMP signaling on HUVEC cells. 15 min PRF exposure produced more than a 3-fold increase in cGMP. Use of a CaM antagonist, TFP (trifluoroperazine) abolished the PRF effect, providing further support for $Ca^{2+}$ binding as the PEMF transduction mechanism in endothelial cells. *From Wu et al., 2010, with permission.*

A recent randomized blind animal study showed that a PRF waveform, chosen *a priori* using the ECM/SNR model to modulate CaM/NO/cGMP signaling, was able to significantly increase angiogenesis *in vivo* (+ 150%) 7 days following a thermal myocardial injury in the rat. An antibody to CD-31 was employed to identify newly formed blood vessels in tissue sections throughout the penumbra surrounding the ischemic core of the lesion. The PRF effect was eliminated in rats that received L-NAME, a non-selective cNOS inhibitor, in the drinking water, providing strong support that this PRF waveform modulated angiogenesis through NO/cGMP signaling [Strauch et al., 2006; 2009]. This result is summarized in Figure 7.

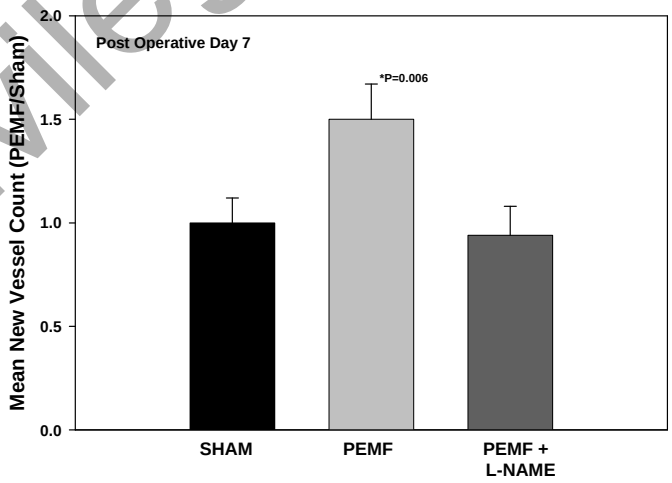


**Figure 7:** PRF increases angiogenesis by 60% in a thermal myocardial necrosis model in the rat. L-NAME, a general cNOS inhibitor fed to rats in the drinking water, eliminated the PRF effect on angiogenesis, providing strong support that $Ca^{2+}$ binding to CaM in the NO signaling cascade is the EMF transduction mechanism (see fig 1). *From Strauch et al., 2009, with permission.*

**Neurons**

In vivo studies have demonstrated that PEMF signals enhance peripheral nerve regeneration, however only two have suggested that CaM-dependent NO signaling may be involved [Walker et al., 2002; Kim et al., 2002]. We have reported that a PRF signal, configured *a priori* using the ECM/SNR model to modulate CaM-dependent signaling, applied for 30 minutes to the MN9D dopaminergic neuronal cell line, increased NO production by several-fold in a serum depletion paradigm and produced a 45% increase in cGMP [Casper et al., 2008]. The PEMF effects on NO and cGMP were inhibited by the CaM antagonist W-7, providing support that the PEMF transduction mechanism proposed here also applies to neurons. These results are summarized in Figure 8.

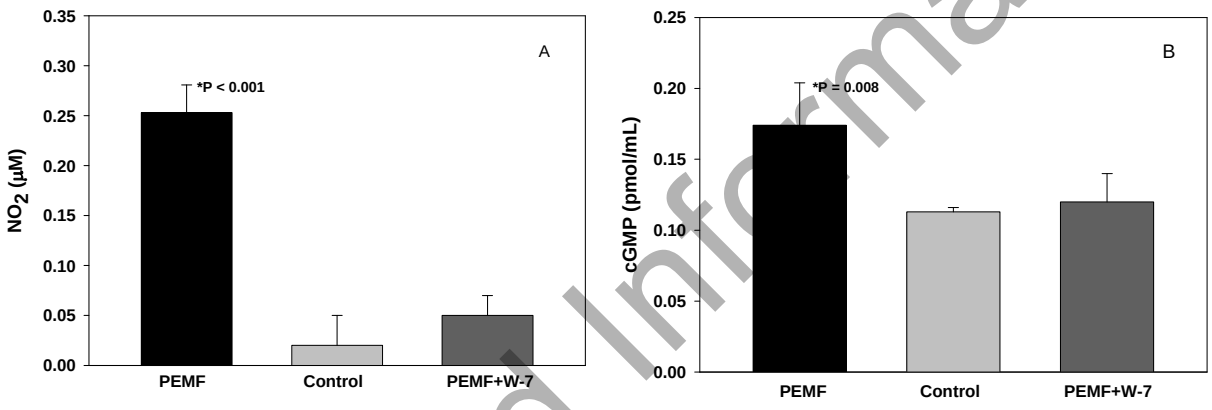


**Figure 8:** MN9D cultures were incubated to serum-free medium in presence or absence of the CaM antagonist, W-7. After 30 min exposure to a PEMF signal configured to modulate the CaM/NO signaling pathway, NO was quantified in conditioned medium (Griess reaction), and cell lysates were analyzed for cGMP (ELISA). PEMF increased NO several-fold over that from control cultures (**A**), which was blocked by W-7. Levels of cGMP by increased 45% (**B**), which was also obliterated by W-7, supporting application of the PEMF transduction mechanism proposed here to neurons.

Cyclic AMP is known to play a significant role in differentiation and survival [Siddappa et al, 2009], and given the relatively small PEMF effect on cGMP as described above, we decided to assess the effect of the same PRF signal on cAMP production in MN9D cells (see Part II of this series). A summary is presented here. MN9D cells in serum free medium were removed from the incubator (repeatable temperature stress injury to transiently increase intracellular $Ca^{2+}$) and exposed to a PRF signal, configured to modulate the CaM/NO signaling pathway, for 15 min. cAMP was evaluated in cell lysates by ELISA. Results demonstrate that this PEMF signal increased cAMP production by several-fold. Notably, the c-NOS inhibitor L-NAME abolished the PEMF effect on cAMP. The results, summarized in Figure 9, suggest the proposed PEMF transduction mechanism may be applicable to neuronal differentiation and survival.

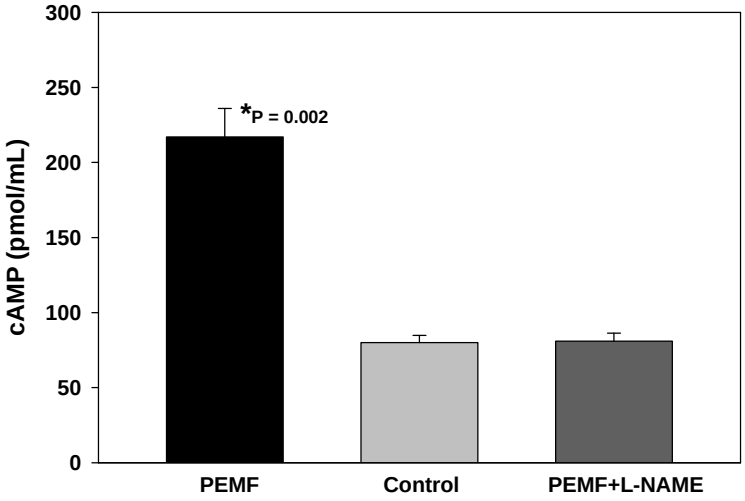


**Figure 9:** MN9D cultures were exposed to serum-free medium in presence or absence of the cNOS inhibitor, L-NAME. After 15 min exposure to PEMF, configured to modulate the CaM/NO signaling pathway, cAMP was quantified in cell lysates by ELISA. PEMF increased cAMP several-fold over control cultures; this increase was completely blocked by L-NAME, supporting application of the PEMF transduction mechanism proposed here to neuronal cells.



## Clinical studies

Evidence that the PEMF transduction mechanism proposed here can lead to significant clinical applications is demonstrated in a recently reported randomized, placebo-controlled, double-blind clinical trial [Rohde et al., 2009]. In that study a radio frequency PEMF signal, configured *a priori* using the ECM/SNR model to modulate the CaM/NO signaling pathway, was applied immediately after breast reduction surgery in human subjects. Wound exudates were collected and pain was assessed by participants using a validated Visual Analog Scale (VAS). Concentrations of IL-1β, a major pro-inflammatory cytokine, were approximately 3-fold lower at 5 hours post-op ($P < 0.001$) compared to those of the control group in wound exudates from PEMF-treated patients. PEMF also produced a concomitant 2-fold decrease in pain at 1 hour ($P < 0.01$) and a 2.5-fold decrease at 5 hours post-op ($P < 0.001$), persisting to 48 hours post-op. No significant changes in VAS scores were observed in the control group. Furthermore, the increased levels of analgesia were reflected in a 2.2-fold reduction in narcotic use in patients receiving active treatment over the first 24 hours post-op ($P = 0.002$). Importantly, the time course for both pain and IL-1β reduction were concomitant, suggesting that PEMF, configured to modulate CaM/NO signaling, produced endogenous changes in the dynamics of IL-1β availability, which would impact the many known subsequent inflammatory events that are mediated by this cytokine [Ren and Torres, 2009], including those leading to post-operative pain. These results are summarized in Figure 10.

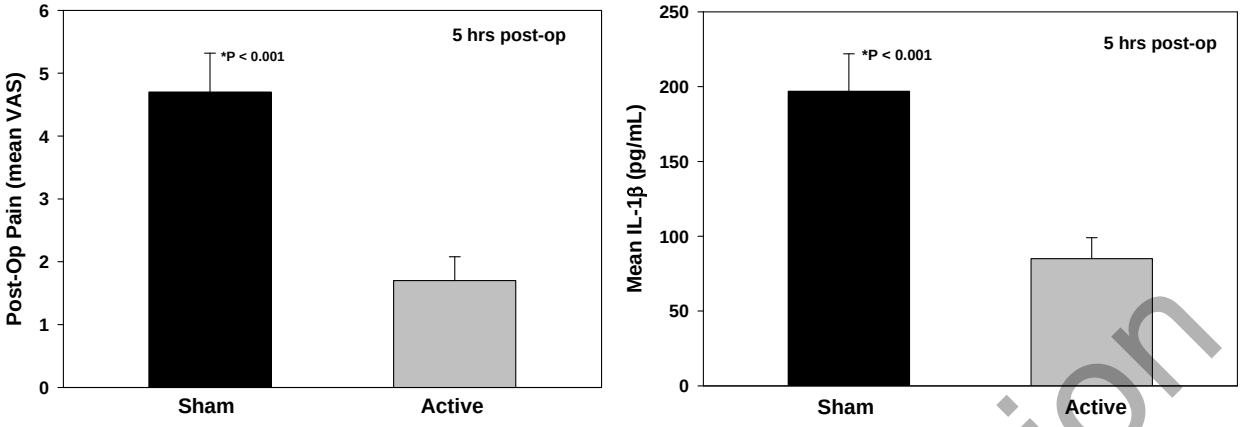


**Figure 10:** Effect of a radio frequency PEMF signal configured to modulate CaM/NO signaling in a randomized double-blind clinical study on breast reduction patients. Pain was approximately 2.5-fold lower in the active cohorts by 5 hrs post-op. A measure of the cytokine, IL-1β in wound exudates showed nearly the same 2.5 fold decrease in the active cohort over the same time range, providing strong support that actions of PEMF were mediated through the CaM/NO signaling pathway. *From Rohde et al.,2009, with permission.*



Others have reported that the CaM/eNOS/NO signaling pathway down-regulated levels of both IL-1β and iNOS [Ren and Torres, 2009; Palmi and Meini, 2002], and importantly, that PEMF down-regulated iNOS at the mRNA and protein levels in monocytes [Reale et al., 2006] and decreased IL-1β and TNF-α in fibroblast-like cultures [Gomez-Ochoa et al., 2010]. Because the PEMF signal used in this clinical study was identical to that which has been shown to modulate CaM-dependent NO signaling, it is probable that PEMF down-regulated both IL-1β and iNOS, which led to post-operative analgesia. These results are similar to those obtained earlier for the effect of the same PEMF signal on post-operative pain in a randomized double-blind study in breast augmentation patients [Hedén and Pilla, 2008]. In that study a reduction in pain occurred nearly 3-fold faster in subjects exposed to PEMF signals by post-operative day 2, with a concomitant decrease of nearly 2.5-fold in narcotic pain medication.

In addition to its effects on pain from surgical incisions, PEMF signals can produce analgesia in more chronic conditions that produce damage to other tissue types. A randomized double-blind clinical study demonstrated that a PEMF signal configured *a priori* to modulate the CaM/NO signaling pathway using the ECM/SNR model produced a rapid decrease in pain from osteoarthritis of the knee [Nelson et al., 2010]. A total of 37 patients (19 active, 18 sham) entered the study. A PEMF signal, configured, *a priori*, to modulate $Ca^{2+}$ binding to

CaM consisting of a 7 msec burst of 6.8 MHz sinusoidal waves repeating at 1/sec with 0.05 G peak amplitude was used for 15 minutes twice daily, or as needed for pain relief. Maximum VAS scores were obtained at baseline (day 0) and daily for the first 14 days and from day 29 to day 42. Results show that PEMF caused an approximately 2-fold decrease in mean VAS with respect to baseline by the end of day 1 in the treated group, which persisted to day 42 ($P < 0.001$). There was no significant decrease in mean maximum pain scores at any time point in the sham group. Representative results are summarized in Figure 11.

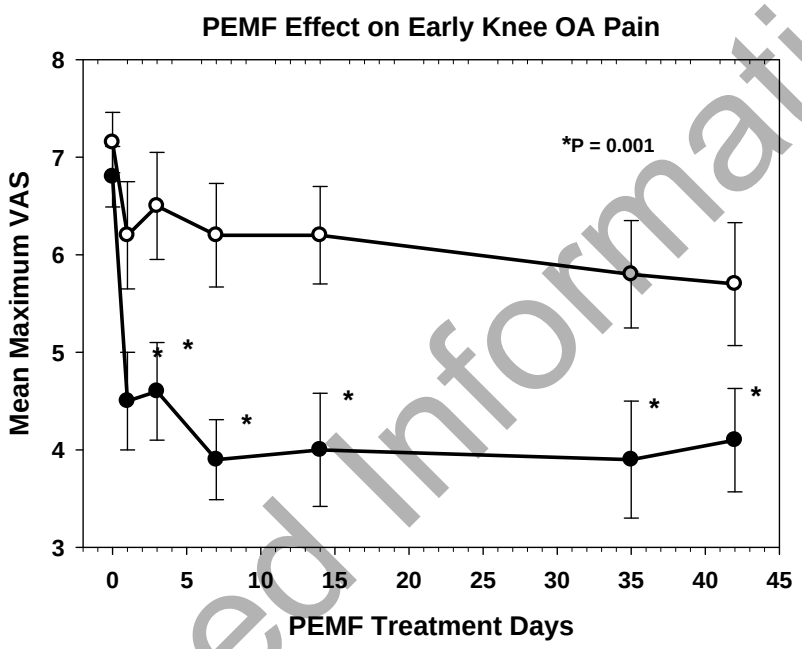


**Figure 11:** Effect of radio frequency PEMF, configured to modulate CaM/NO signaling, on pain from knee osteoarthritis in a randomized double-blind clinical study. Mean maximum pain (VAS) in the active cohort decreased by 2-fold compared with its baseline value within the first 24 hours, which persisted to day 42. There was no significant decrease in pain in the sham cohort at any time point.. *From Nelson et al., 2010, with permission.*

It has been proposed that CaM-dependent NO production rapidly mediates relief of pain from knee OA by increasing circulation, decreasing nerve irritation, and decreasing inflammation [Torres et al., 2006]. This is supported by similarities in the kinetics of the rapid reduction of pain in the PEMF group in the OA study to that in the breast reduction study mentioned above, wherein PEMF also produced a rapid initial reduction in pain within 5 hours post-op. This suggests that the mechanisms of action are similar in both studies. If that were the case, then PEMF would have been expected to activate the CaM/cNOS pathway producing an initial rapid and transient release of NO leading to vaso- and lymphatic dilation. This could

cause a rapid reduction of bone marrow edema with a concomitant rapid reduction of pain. It is also probable that the mechanism of the PEMF effect involves the down-regulation of IL-1β with its consequent attenuation of inflammation in this patient population.

**DISCUSSION**

Ever since biological effects from non-thermal electromagnetic fields were first reported, many studies have attempted to show that PEMF, from ELF to RF, caused increases, decreases or oscillations in cytosolic concentrations of $Ca^{2+}$ [McCreary et al., 2006; Marchionni et al., 2006; Platano et al., 2007]. However, many such studies have either been equivocal or failed to demonstrate any PEMF effect. In contrast, the transduction mechanism proposed here predicts that a PEMF effect occurs only when intracellular $Ca^{2+}$ homeostasis has been disrupted, and the PEMF signal has frequency content of sufficient amplitude within the bandpass of Ca/CaM binding kinetics to be detectable above thermal noise. Thus, in the absence of an appropriate insult, injury, or imbalance affecting cytosolic $Ca^{2+}$ homeostasis, the EMF-sensitive pathway remains quiescent. According to the PEMF transduction mechanism proposed here, changes in free cytosolic $Ca^{2+}$ is a signal for endogenous tissue repair and regeneration mechanisms, which opens the PEMF-sensitive pathway (Fig. 1). Quiescent cells, in which there is no transient increase in cytosolic free $Ca^{2+}$ caused by injury or insult do not appear to respond to PEMF in a physiological significant manner, providing one explanation for the reports of no known side effects from current therapeutic applications of PEMF.

Notwithstanding the above, several studies have shown that quiescent cells and tissues can respond to PEMF by producing heat shock proteins. This is consistent with the proposed PEMF transduction mechanism because $Ca^{2+}$ binding is voltage-dependent and kinetically asymmetrical, with the forward reaction favored. In fact, PEMF signals, if configured as described in this study, may slightly disturb homeostasis and activate CaM even when cytosolic $Ca^{2+}$ concentrations are at baseline. Furthermore, there is evidence that NO induces the expression of HSP72 [Li et al., 2008]. NO is also bimodal in this pathway, increasing HSP expression from prolonged exposure, as can occur if iNOS remains up-regulated in an uncontrolled fashion. It follows that, if a PEMF signal contains frequency components of sufficient amplitude within the bandpass of Ca/CaM binding, PEMF can modulate HSP expression via CaM-dependent NO signaling, even for quiescent cells and tissues. Once expressed, HSP can be released extracellularly, whereupon it will bind to the surfaces of

adjacent cells and initiate signaling cascades or enter the bloodstream (HSP60 and HSP70) and bind to distant cell targets [Calderwood et al., 2007]. Release of HSP prior to injury can reduce the inflammatory response because it is poised to rapidly downregulate IL-1β and iNOS [De Paepe et al., 2009], which would protect tissue from injury. Several studies have described the effect of pre-treatment with PEMF in an ischemia/reperfusion model [DiCarlo et al., 1999; Ronchi et al., 2004; George et al., 2008]. Although there is not yet any supporting data, it is reasonable to speculate that the effect of PEMF on post-surgical pain and edema reported here included a contribution from HSP.

Perhaps the strongest evidence supporting the proposed PEMF transduction mechanism is provided by the CaM antagonist studies at the cellular level. Referring to the mechanism schematized in Figure 1, it is clear that CaM is activated by binding cytosolic $Ca^{2+}$, which is voltage-dependent, and has a rate that places its bandpass in a much higher frequency range than that for the remaining steps in this signaling cascade. It is also clear that the biological signaling cascade depends upon the activation of CaM. Thus, prevention of activated CaM from binding to cNOS will stop biological signaling upstream and close to the first step in the pathway. Results from cell-free CaM-dependent enzyme studies also suggest that the PEMF effect on CaM activation is $Ca^{2+}$ - dependent. With this in mind, nearly every one of the cellular studies reported here showed that inhibition of activated CaM from binding to cNOS eliminated the PEMF effect on subsequent steps in the biological signaling cascade, as well as on the biological endpoint, such as proliferation.

When taken together, this collective evidence strongly supports the role of Ca/CaM-dependent NO production as an important mediator of EMF signaling for cell proliferation and differentiation, as well as tissue repair. The experimental results demonstrate that PEMF signals configured *a priori* with the ECM/SNR model to increase the rate and amount of $Ca^{2+}$ binding to CaM will modulate cNOS activation, producing a fast and transient increase in NO production in endothelial and other non-immune cell types. The actions of NO upon healing or regenerating tissue include the down-regulation of inflammation by the following mechanisms: i) modulation of vasodilation and lymphatic drainage; ii) down-regulating iNOS activity, thus inhibiting the production of inflammatory levels of NO; and iii) decreasing the availability of pro-inflammatory cytokines that are induced by high sustained levels of NO.

Nitric oxide signaling can have additional actions during the healing process. For wound

healing, NO from cNOS can increase cGMP production within minutes, increasing levels of growth factors, such as FGF-2 for angiogenesis and fibroblast proliferation, depending upon the stage of healing. NO from cNOS can also modulate cAMP production, which will orchestrate neuronal cell differentiation and survival.

The ECM/SNR model is useful, not only for the *a priori* configuration of PEMF waveforms to modulate specific biological signaling, but also for the *a posteriori* analysis of any PEMF waveform to assess its bioefficacy. For example, the ECM/SNR model was employed in a recent study of the effect of PEMF on peripheral nerve regeneration [Walker et al., 2002]. In that study, a sawtooth-type waveform, which had been reported to accelerate neurite outgrowth in a culture system, had no effect on nerve conduction or recovery of function after sciatic nerve crush injury in an animal model. In contrast, a previous study, using a different PEMF signal in the same animal model showed that PEMF accelerated recovery [Walker et al., 1994]. Application of the ECM/SNR model to the CaM/NO signaling pathway, known to modulate nerve regeneration and consideration of target geometry, provided the explanation for the differing PEMF effects. The PEMF signals used in the two animal studies had large differences in frequency components. Thus, the relatively narrow, asymmetrical, induced electric field waveform with a burst width of 300/1000 ms, an amplitude of 3mT, repeating at 2 Hz, used in the second study produces substantially less amplitude at frequencies matching $Ca^{2+}$ binding kinetics compared to that produced by the signal utilized in the previous study, which consisted of a symmetrical bipolar pulse, 10 ms/10 ms, 0.3mT, at 2 Hz. Additionally, the size of the culture dish target was about 10 times larger than the sciatic nerve *in vivo*. The result is that the SNR comparison for the signals used in these animal studies was similar to that shown in Figure 3 (lower curve), illustrating that this PEMF signal was mismatched to the CaM/NO signaling pathway.

The ECM/SNR model is also useful to predict whether non-thermal bioeffects may be produced from the RF signals emitted by cellular phones. It is reasonable to assume that $Ca^{2+}$ binding in the reaction scheme (Figure 1) is a potential target for a GSM signal. Thus, the rectifier-like property of Ca/CaM binding in the CaM/NO signaling pathway provides a sufficient condition for a non-thermal RF effect to occur. The necessary condition for a non-thermal bioefffect is that the GSM signal produces sufficient SNR in the $Ca^{2+}$ binding target. This has been evaluated for a single 577 μsec burst of an 1800 MHz GSM signal and a single 2000 μsec burst of a 27.12 MHz sinusoidal PRF signal, configured to modulate the CaM/NO

signaling pathway [Pilla and Muehsam, 2010]. The results (Figure 12) show that SNR for a single 577 μsec pulse of 1800 MHz the GSM signal at 20 V/m is similar to that of a single 2000 μsec pulse of the 27.12 MHz PRF signal at 12 V/m, typical measured field strengths within the biological target for these signals. As may be seen, peak SNR for the GSM signal is in the same range as that for the PRF signal and occurs in a frequency range consistent with published values for $k_{on}$ for Ca/CaM. Therefore, the GSM signal may be expected to modulate the CaM/NO signaling pathway, as shown throughout this study.

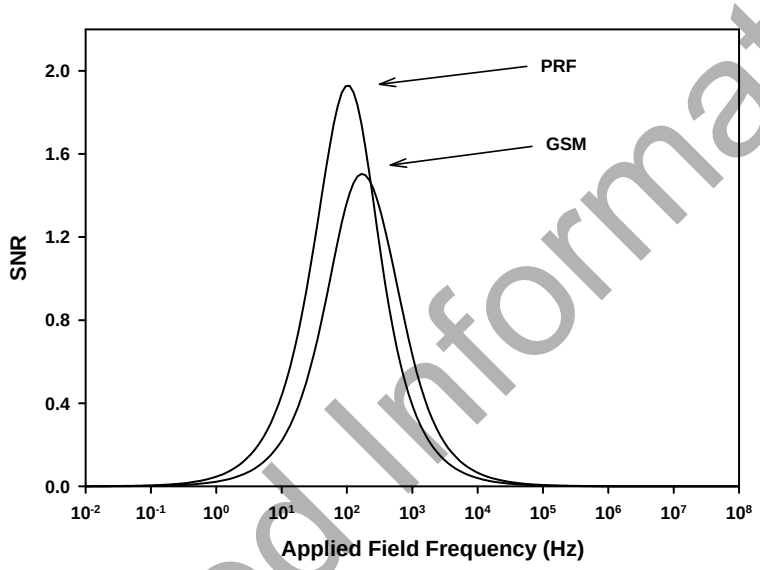


**Figure 12:** Comparison of SNR in the Ca/CaM binding target for a PRF signal consisting of a single 2000 μsec burst of 27.12 MHz RF carrier, with that for a single 577 μsec burst of an 1800 MHz GSM signal. SNR is similar for both signals and occurs in a frequency range consistent with Ca/CaM binding kinetics. Both RF signals may, thus, be expected to yield similar effects on the CaM/NO signaling pathway.



This analysis suggests that it is incorrect to assume that GSM mobile phone signals are incapable of producing non-thermal bioeffects. On the contrary, this SNR comparison predicts that a GSM signal could have effects on signaling pathways critical to a variety of growth and repair cascades in the presence or absence of detectible tissue heating. Furthermore, because therapeutic PRF signals have no known side effects, it is unlikely that GSM signals will produce undesired bioeffects other than those due to excessive tissue heating. Thus, in the appropriate context, GSM signals may have therapeutic effects, as suggested by a recent report showing that long-term exposure to a GSM signal protects against and reverses cognitive impairment in a mouse model of Alzheimer's disease

[Arendash et al., 2010]. Indeed it has been suggested that inflammation, mediated by both IL-1β and iNOS enhance the deposition of β-amyloid [Chiarini et al., 2006]. According to the transduction mechanism proposed here, a GSM signal would be expected to down-regulate both factors, which may prevent or reverse the effects of Alzheimer's disease and any other neurodegenerative disease with an inflammatory component [Casper et al., 2000; McGeer and McGeer, 2004].

Finally, it is important to emphasize that the EMF transduction mechanism as presented here applies only to *in situ* electric field effects on the voltage-dependent ion-binding step in biological signaling processes. However, there are many reported bioeffects from weak DC and combined DC/AC magnetic (B) fields. For example, several groups have shown that weak B-fields can modulate CaM-dependent enzyme activity [Markov et al., 1993; 1994; Markov and Pilla, 1997; Engstrom et al., 2002; Liboff et al., 2003]. Other groups have shown that low frequency B-fields affect NO signaling [Patruno et al., 2010; Reale et al., 2006]. Of the many models proposed to explain the bioeffects of weak magnetic fields, those involving modulation of the bound trajectory of a charged ion by classic Lorentz force [Edmonds, 1993; Zhadin, 1998; Muehsam and Pilla, 1996; 2009a; 2009b; Pilla et al., 1997] are most relevant to the reaction scheme presented in Figure 1, suggesting that the trajectory of the ion within the binding site itself could affect reactivity. One interpretation of this is that weak DC and certain combinations of weak AC/DC magnetic fields could enhance or inhibit the exit (unbinding) of the target ion, thereby accelerating or inhibiting the overall reaction rate by manipulating $k_{off}$, even in the presence of thermal noise. Application of the Lorentz force model, *a posteriori*, to CaM-dependent myosin phosphorylation [Muehsam and Pilla, 2009b] provides convincing support for the proposal that weak magnetic fields could also affect biological signaling via modulation of $k_{off}$ according to the overall EMF transduction mechanism schematized in Figure 1.



**CONCLUSIONS**

This study presents a model and supporting experimental evidence that an EMF signal can be configured *a priori* to act as a first messenger in CaM-dependent signaling pathways relevant to tissue growth, repair and maintenance. In the cases considered here the second messenger is free cytosolic $Ca^{2+}$, for which CaM is a primary responder when homeostasis is disrupted. Thus, the proposed EMF-sensitive pathway is normally quiescent and is activated only when $Ca^{2+}$ homestasis is disturbed. The kinetics of $Ca^{2+}$ binding to CaM are asymmetrical because

binding takes place orders of magnitude more rapidly than release. Such kinetic asymmetry gives Ca/CaM binding rectifier-like properties, suggesting that any bipolar PEMF signal for which the pulse duration or carrier period is significantly lower than the bound lifetime will drive voltage-dependent binding. This is, then, a general transduction mechanism by which non-thermal PEMF will modulate the response to any insult that causes concentrations of intracellular free $Ca^{2+}$ to rise in any tissue. Application of this mechanism to tissue repair requires that intracellular $Ca^{2+}$ homeostasis be disrupted by injury before a PEMF effect can take place, providing a convincing explanation for the lack of known side effects from PEMF therapy.

The ECM/SNR model is powerful enough to unify the observations of many groups and to offer a means to explain both the wide range of reported bioeffects as well as the many equivocal reports from PEMF studies. Knowledge of the kinetics of $Ca^{2+}$ binding to CaM allows the use of an electrochemical model by which the ability of any PEMF signal to produce a net increase in bound $Ca^{2+}$ can be assessed. *In vitro* studies using antagonists and inhibitors provide strong support that PEMF could indeed be configured to modulate CaM-dependent NO signaling. Results from clinical studies using such PEMF signals provide further support. These same signals may slightly disturb homeostasis and activate CaM even when cytosolic $Ca^{2+}$ concentrations are low because $Ca^{2+}$ binding is voltage-dependent and the forward reaction is favored. This, in turn, will effect the release of CaM-dependent HSP into the blood stream, which suggests that PEMF can produce significant prophylactic bioeffects. These predictions open a host of significant possibilities for clinical and wellness applications that can reach far beyond relief from post-operative pain and edema.

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