Effects of a low-intensity electromagnetic field on fibroblast migration and proliferation.

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Abstract

The aim of this study was to test if an extremely weak 1GHz electromagnetic field (EMF), known to be in resonance with clusters of water molecules, has biological effects on human fibroblasts. We demonstrate that in an in vitro model of wound healing, this EMF can activate fibroblast migration. \[^{3}\text{H}]\text{thymidine incorporation experiments demonstrated that the EMF could also activate fibroblast proliferation. Activation of the expression of human fibroblast growth factor 1 (HFGF1) after EMF exposure showed that molecular wound healing pathways are activated in response to this water-resonant EMF.}
A fibroblast is a type of cell that synthesizes extracellular matrix and collagen, the structural framework for animal and human tissues, and plays a critical role in wound healing. Fibroblasts are the most common cells of the connective tissue in animals. The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix, and as a result this cell type is critically involved in the process of wound healing. Fibroblasts begin to enter a wound site two to five days after wounding as the inflammatory phase ends, and their numbers peak at one to two weeks post-wounding. By the end of the first week, fibroblasts are the principal cell lineage in the wound, and they are the main cell type that lays down the collagen matrix in the wound site [Stadelmann and others 1998]. Collagen deposition is important because it increases the strength of the repairing wound; in addition, cells involved in the regulation of inflammation, angiogenesis, and further connective tissue construction attach to, proliferate and differentiate on the collagen matrix laid down by fibroblasts [Ruszczak 2003].

It is known that EMFs play an important role in the cascade of processes determining cell migration, adhesion and differentiation. The electrical currents the related fields are generated by passive Na\(^+\) uptake from the environment leading to an internally positive transepithelial potential difference (TEP) [Funk and Monsees 2006]. Endogenous EMFs also exist in the immediate vicinity of wounds, where they are created due to a disruption of the TEP in the epithelial layer. This electric potential collapses at the wound site, but rises to the potential of healthy cells with increasing distance from the wound [Song and others 2002]. For this reason, the application of EMFs may have therapeutic relevance for wound healing and other pathologies. Currently studies of the biological effects of EMFs are performed with high-frequency (pulsed) EMFs (1-100 Hz) or with those of low physiological frequencies (8-30 Hz)[Funk and Monsees 2006].
In a number of studies, it has been demonstrated that EMFs of different frequencies can accelerate wound healing [Strauch and others 2007]. The last study employed an industrially-produced therapy device, the action of which is based on an EMF-induced direct current. The authors were able to observe the stimulation of keratinocyte cells participating in wound re-epithelialization, but did not detect significant effects on fibroblast migration or proliferation.

In our investigation, we have studied the biological effects of the developmental therapeutic EMF generator Aquaton-2 (Telemak, Saratov, Russia). This device emits weak radio waves at a frequency of 1 GHz. When placed over cell culture plates, the conical antenna creates a final energy density of 6nW/cm$^2$. It was previously demonstrated that frequencies of 50, 3 and 51, 8 GHz millimeter wavelength and 1GHz decimeter wavelength correspond to the resonant (natural) frequencies of clusters of water molecules, and also to animal tissues which have similar water resonant spectra [Sinitsyn and others 2000]. This resonance interaction with aqueous media is observed at very low powers (below 1 mkW/cm$^2$), so that EMF energy can be transferred between clusters of water through hydrogen bonds without causing heating, and can reach internal areas of tissues and cause biological effects by enhancing the natural oscillations of water molecules, and probably by generation of super-weak currents.

Firstly we studied the effects of the 1 GHz EMF on fibroblasts in a migration assay which has been widely used as an in vitro test of wound healing. It is possible to quantify the speed of fibroblast migration by measuring the area of the artificial in vitro wound (Fig.1a). Human Dermal Fibroblasts (HDFs) (PromoCell, Heidelberg, Germany) were cultured in DMEM supplemented with 10% FBS in twelve-well plates that were pre-coated with collagen (50 μg/ml) and blocked with BSA (3% BSA in PBS). A scratch was performed with a micropipette tip on the confluent cell culture. The relative migration of the cells was calculated using ImageJ software on images taken immediately and 9 hrs after scratching.
To evaluate whether EMF had effects on fibroblast migration, we performed 5 independent experiments in which scratches were generated in 5 wells of two 12-well plates. One of the plates was treated with the EMF, and the other served as the control. The control and experimental plates were removed and replaced in the CO$_2$ incubator simultaneously to avoid the effects of possible differences in environment. The measurement of wound gaps was performed before treatment and after 9 hours of incubation, and demonstrated an acceleration of fibroblast migration of approximately 18% (P=0.017) (Fig. 1b).

We further measured the efficiency of DNA replication which is directly related to cell proliferation. To address the effect of the 1 GHz EMF in the proliferation of fibroblasts, we performed \textit{in vitro} $^3$H-thymidine incorporation assays. Cells were seeded in 35 mm plates and cultured until they reached 70% confluence. Cells were treated for 20 min with the Aquaton-2 device in 5 independent experiments. Five microliters $^3$H-thymidine (1mCi/ml) was then added directly to the incubation media; the cells were incubated for 3 hours and then treated. The cells were then washed twice with ice-cold PBS, followed by two washes with 5% TCA and one wash with PBS. The cells were then solubilized by adding 800 µl 0.5N NaOH /0.5% SDS followed by thorough aspiration. The solubilized cell solution was then collected into 5 ml scintillation vials, 4 ml Ultima gold scintillation cocktail was added, and the vials were counted. Comparisons between $[^3]$H]thymidine incorporation into control and EMF-treated cells demonstrated that HDFs responded to Aquaton-2 treatment with an approximate 22% (P=0.005) stimulation of $^3$H-thymidine incorporation (Fig. 2 a,b).

To examine whether molecular biological effects related to artificial wound healing occur in response to EMF treatment, expression of human fibroblast growth factor 1 (hFGF1) and human vascular endothelial growth factor (hVEGF) were measured before and 1 hour after treatment. cDNA was prepared by reverse transcription of total RNA with SuperScript III,
according to the manufacturer’s protocol (Invitrogen). Quantitative RT-PCR cDNA was performed with primers selected for specificity from the Harvard University primer bank (http://pga.mgh.harvard.edu/primerbank/). Real-time PCR was performed on an Applied Biosystems 7300 instrument using Platinum SYBR Green qPCR Supermix-UDG with ROX reference dye (Invitrogen). Two house-keeping genes, RLP 32 and GNBL2, were used as reference genes. Both FGF1 and VEGF mRNA levels were found to be associated with the EMF treatment. While FGF mRNA levels were significantly increased in fibroblasts treated by the 1 GHz EMF (Fig. 2b), an association between VEGF mRNA and EMF treatment was not significant, although treated cells tended to have higher VEGF mRNA levels than untreated cells (data not shown).

Here we demonstrated the biological effects of a super-weak 1 GHz EMF which has biological effects due to resonance with water clusters. This suggests that a very low intensity EMF can cause significant biological effects. The biological action of EMFs of much higher energies which are used at present in biological studies may mainly be related to effects involving heating and induced direct currents. The idea of using a weak pulsed EMF which can resonate with certain molecular oscillations and can produce biological effects is not new. [Rosenspire and others 2001; Rosenspire and others 2005] employed a weak EMF to the amplitude of NAD(P)H oscillations. We have used a similar approach using EMF resonant to water clusters to evaluate the effects of the EMF on fibroblasts in an in vitro model of wound healing. Recently it was demonstrated that a much stronger EMF could accelerate normal and diabetic wound healing by increasing vascular density. The authors could demonstrate that the EMF could activate the proliferation of endothelial cells [Callaghan and others 2008]. Another study demonstrated that human skin keratinocyte (a key cell type in wound re-epithelisation) migration and proliferation can also be enhanced by a non-invasive EMF [Huo
and others 2009]. The data concerning fibroblasts is more controversial. While Huo e.a. did not find any influence of an EMF on fibroblast migration and proliferation, another study reported the stimulation of human fibroblast migration in response to an EMF [Sun and others 2004]. In the study of Hou, it is hypothesized that the main mechanism of non-invasive EMF influence is the induction of electrical currents in cells surrounding the target cells. It is possible that similar effects are induced by the super-week (6 nW/cm\(^2\)) high-frequency EMF employed in our fibroblast experiments, where enhancement of natural oscillations in water molecules might enhance super-weak currents in cellular structures. FGF1 is involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, cell migration and proliferation. VEGFA stimulates the growth of new blood vessels. The activation of hFGF1 expression, and the tendency for increased VEGFA expression following EMF treatment demonstrated in the current study show that molecular wound healing pathways are activated in response to water resonant EMFs.
References


Legends to the figures.

Fig. 1 Effect of a 1 GHz EMF on fibroblast migration.

A. Representative images of fibroblasts in artificial wound healing experiments taken immediately (left) and 9 hours later after scratching (right). (A microscope with a digital camera was used to capture images)

B. Quantification of fibroblast migration. The “wound” area was calculated in pixels with ImageJ 1.32 Software (National Institute of Health) immediately and 9 hours later after scratching and expressed as percentage of healed wound of the original area (mean ± SEM). *P=0.017.

C. Fibroblast proliferation efficiency based on $^3$H-thymidine incorporation assays measured as counts per minute (cpm) (mean ± SEM). *P=0.005

NTF, Non-treated fibroblasts; EMFTF, Fibroblasts treated by EMF;

Fig. 2 Effect of a 1 GHz EMF on hFGF1(A) and hVEGFA (B) mRNA expression. mRNA expression level was calculated by normalizing average Ct-value of the two reference genes with the experimental genes values (mean ± SEM).

(A) hFGF1 mRNA expression in skeletal muscle. *P=0.012

(B) hVEGFA mRNA expression in skeletal muscle. P=0.118
Fig. 2

A

B