

Antimicrobial Effects of Electromagnetic Fields: A Review of Current Techniques and Mechanisms of Action

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Considering the worldwide emergence of antibiotics resistance, developing non-drug antimicrobial and antibacterial treatments are necessary. Electromagnetic fields (EMFs) have shown antimicrobial effects in different frequencies and intensities. So far, different modalities of EMFs showed antimicrobial and antibacterial effects in different pathogens. Electric fields, magnetic fields and pulsed EMFs (PEMFs) are common techniques showing promising antimicrobial effects. Despite the various studies indicating the antimicrobial effects of EMFs, the mechanisms of actions of them are not yet completely understood. The present study reviews the most current techniques of EMFs in antimicrobial studies and mechanisms of actions of these methods. Systematic review of studies published on the antimicrobial effects of EMFs in PubMed and Medline is performed. The efficacy of each technique and mechanisms of action were reviewed. Static magnetic field and PEMFs show promising antimicrobial effects for some of common bacterial pathogens. These treatments can be developed as alternative or at least as an adjunctive treatment for some infectious diseases and wound. Further controlled studies are needed to develop new techniques based on EMFs for microbial infections.

Key words: Antibacterial Effects, Electromagnetic Fields, Mechanism of Action, Antimicrobial Effects, Treatment.

Since the 18th century scientists have been intrigued by the interaction of electromagnetic fields (EMFs) and various life processes. So far, plenty of bio-effects of electric, magnetic, and electromagnetic fields on human beings, animals, cells and homogeneous enzyme reactions have been described either in vitro or in vivo conditions¹⁻⁵. EMFs have reportedly therapeutic potentials for a wide variety of diseases including musculoskeletal diseases⁶, cancer treatment⁷, neurological disorders^{7, 8}, wounds⁹⁻¹¹.

During the last two decades, there has been a surge increase in the research interest to the biological interactions and potential therapeutic avenues for EMFs. The studies on

the EMFs biological interactions have focused on different fields. The subjects and number of the studies presented in the 3rd international workshop on the biological effects of EMFs provide a good clue (Greece, 2004)¹². From 192 papers published in the proceeding fields of the conference, 93 explained experiments with EMFs (static, low frequency or radio frequency) and living systems. Nearly 68% of the papers using low-frequency fields reported significant effects on the exposed organisms. This "static" is only an approximation; it does not explain the strength of the effects but it shows that there is no little effect of EMF. 23% of the experimental works studied brain activity and nerve systems, the object of 15% was epidemiology and 13% studied the effects on tumors and clinical applications of EMFs in medicine¹².

In recent years, scientists have attempted to find out whether such fields can affect living organisms. First, they focused on the epidemiology

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and the connection between power-lines and human tumors and leukemia. Later, the research turned to the effects of EMFs on the molecular and cellular level¹²

Objects studied were cells¹³, tissue¹⁴, and whole living organisms^{15, 16}. The viability and proliferation¹⁷, activity of enzymes¹⁸, transport of ions¹⁹ and gene transcription or expression^{20, 21} were investigated with different results.

Markov *et al*²² concluded that it is important to explore exposed organism not only on the cellular or tissue level but also on the complex effects on the whole organism. Accordingly, bacteria^{2, 23}, or yeast²⁴⁻²⁶ – unicellular organisms – are interesting research topics for the study of electric fields (EFs), magnetic fields (MFs) and EMFs effects.

Previous studies have shown that electrical fields can heal the nonunion fracture as effective as bone grafting alone, depending on the anatomical site and degree of nonunion²⁷. Improved success rates have also been reported when exposure to EMFs is coupled with surgical intervention^{28, 29}.

A host of attempts to explain MFs effects on the molecular level have been made², demonstrating that MFs can affect biological functions of organisms through modulating the concentration of hormones, the activity of enzymes or the transport of ions by cell membranes, and also the synthesis or transcription of DNA³⁰⁻³².

Electromagnetic (EM) waves are time varying electric and MFs that propagate at different frequencies (energies) and the biological effects vary with frequency. The most energetic ‘ionizing radiation’, such as cosmic and X-rays (10¹⁸-10²² Hz) damage cells and even much lower frequencies of ultraviolet (10¹⁶ Hz) waves can damage skin. Lower frequency waves are ‘non-ionizing’, but microwaves (10⁹-10¹¹ Hz) that cook foods obviously are harmful to the living organisms.

This paper aims to review the current applications of EMFs as antimicrobial and especially antibacterial treatment and also their mechanisms of action. The physical interactions of static magnetic field (SMF), static electric field (SEF)³³, and EMFs with bacterial and microbial agents are scrutinized to sketch their backgrounds and principal procedures and to compare their antimicrobial performance.

Static Magnetic Fields

The biological effects of SMFs and SEFs are different from EMFs, combined electric and MFs. Therefore, scientists have been interested in investigating how a SMF interacts with living organisms. Indeed, exposure to high-intensity MFs is on the rise because of the widespread use of magnetic resonance imaging (MRI) for medical diagnosis, and nuclear magnetic resonance (NMR) and electron spin resonance (ESR) for instrumental analysis. In order to find the biological effects of SMFs on living systems, it is useful to classify SMFs as weak (<1mT), moderate (1 mT to 1 T), strong (1-5 T) and ultra strong (>5 T)³⁴. Scientists have been interested in assessing the effect of moderate and strong intensity SMFs on living organisms.

According to recommendations from the European Union (EU), SMFs below 0.5T are commonly considered quite secure for humans and no permission is necessary for installation and use of machinery with fields below 0.5T, such as in MR tomography. The mechanisms by which MFs influence biological material are poorly understood³⁵

High intensity SMFs are widely used in medical and research laboratories such as magnetic resonance imaging (MRI) and nuclear magnetic resonance (NMR). In recent years, peruses of the biological effects of strong MFs have been intensified due to their possible harmful or useful effects on many eukaryote organisms, including human beings³⁶⁻³⁸. As relatively simple living organisms, bacteria are important research subjects in this field.

SMF can be produced by magnetic material or magnetic disks etc.

Cell Growth and Viability Research relies on CFU as a touchstone for cell growth.

Bellia *et al* (2004) studied and compared the effects of to 50 HZ MF with intensity 0.5 mT and the SMFs in the range of 0.1-100 mT on *Saccharomyces cerevisiae* strains. The assessment measure was duplication time. They found that there was no significant difference between the samples were exposed and not exposed. But the experimental error for these measurements was 28% and the experiment was not able to detect smaller changes in the growth of the yeasts. It seems that colony- forming units (CFU) counting is a better

technique to assess the growth rate of *S. cerevisiae*³⁹.

Wenjin *et al* (2009) used SMFs on *E. coli*. The experiments suggested that the SMF inhibited the growth and propagation of *E. coli* cells greatly or even killed a large number of the cell during the initial stage of SMF treatment⁴⁰. Moreover, wenjin *et al* found that the *E. coli* cells were most sensitive to the SMF at higher temperature because the relative number of CFU decreased with increasing temperature. The results could be interpreted with membrane theory^{34,41-45}. This theory expresses that, the diamagnetic properties of membrane phospholipids determine the SMF's effects on living organisms. The reorientation of these molecules during SMF's exposure will result in the deformation of imbedded ion channels, thereby altering induced rotational excitation of the hydrocarbon chain that occurs and this makes the reorientation of the molecules much easier. Therefore the SMF's effects on organisms are enhanced.

Weimin *et al* (2005) found that 12h exposure of the 14.1 T MF has no detectable effect on the cell growth of *S. oneidensis*⁴⁶ and this result was different from the result Horiuchi *et al.* (2001) on *E. coli* cultured. Horiuchi *et al.* (2001) found that the number of viable cells of *E. coli* B in the stationary phase after 48 h under the MF of 5.2–6.1 T was 100 000 times higher than that under a geomagnetic field⁴⁷.

Kohno *et al* (2000) explored the effect of SMF on some culture of such as bacteria *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*. They reported that when cultured under anaerobic conditions, the ferrite magnet caused strength-dependent decreases in the growth rate and maximum number of bacteria for *S. mutans*, *S. aureus*, but their growth was not inhibited under aerobic conditions. The results indicated that the *S. mutans* and *S. aureus* growth is dependent on oxygen⁴⁸.

Stansell *et al* (2001) reported that SMF can lead to significant increase the antibiotic resistance of *E. coli*⁴⁹.

TEM and SEM Assessments

In order to identify why the SMFs can affect the viability of *E. coli* cells, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are used⁵⁰. These two

instruments showed that the cell surface was damaged while exposed to SMFs. The untreated cells surface was smooth while the treated cells surface was broken at the cell two ends. This might be caused by strong oxidation effect of oxygen free radicals which were produced by SMFs treatment^{48, 51}. There were three main theories to explicate the effect of SMFs on living organisms: (1) Ion interference mechanism (paint): SMF affects the binding state of ion–protein complex. This theory indicates that the SMF does not cause any quantum transitions. It is just an interference effect of long-lived quantum states of the ion within the protein capsule. (2) Free radicals theory^{48,51}: when the bacterial solution is exposed to the SMF, it created oxygen free radicals. These free radicals include H_2O^{\bullet} , O^{2-} , $\bullet OH$, H_2O_2 , etc. These compounds are highly reactive so that can cause great damage to the cells of living organisms. (3) Membrane theory: plasma membranes of cells are composed of diamagnetic anisotropy molecules. In the presence of SMF, the molecules will rotate and ultimately achieve an equilibrium orientation, representing the minimum free-energy state. The molecular rotation within the membrane matrix will influence imbedded ion channels and therefore affect the ions mobility.

The phenomenon observed in the experiments described suggests that the effect of SMFs on the bacterial strain may be interpreted by a combination of these three theories.

Kohno *et al* (2000) found that if MFs decrease dissolved oxygen and to $\bullet OH$ synthesis, and if we assume that the MF action is related to the behavior of the oxygen and active oxygen, active oxygen formation may be induced by MFs⁴⁸. Involvement of nitrogen oxide (NO) as a substance that controls cell membrane channels is also possible⁵².

Gene Expression

To investigate the effects of strong SMFs on gene expression: Tsuchiya *et al* (1999) and Horiuchi *et al* (2001) found that the *rpoS* gene, which encodes a sigma factor and plays a role as a transcriptional regulator of some genes, had increased activities in stationary stage^{47,52}. Gao *et al* (2005) reported that the activities of other transcriptional regulators were affected by strong SMFs under log phase stage of bacterial growth⁴⁶. However, the mechanism underpinning such

expression alterations is not clear.

Low Frequency Magnetic Field

One of the current and most useful methods to investigate antibacterial effects of MFs is to use low frequencies especially frequencies ranging 50 Hz to 60 Hz.

Various studies have been published on the effects of these fields⁴⁵. However, contrary to the publications claiming the bio-effects of EMFs; plenty of studies have shown no significant effects on the living organisms. Lopucki *et al* (2005) reported that no change in oxidative DNA damage after 50 HZ MF exposure was found⁵³.

Dependence of CFU on Exposure Time

Strašák *et al* (2001)⁴⁵ and Fojt *et al* (2003)⁵⁴ investigate the effects of low frequency on bacteria and they reported that the number of CFU decreases with the time of exposure and they found 20% decrease in CFU number for Gram-positive and 30% decrease in CFU number for Gram-negative Fojt, Klapetek⁵⁵.

Falone *et al* (2007) used extremely low frequency (ELF) EMF, 50 Hz, in neuroblastoma cells and it was found that low frequency magnetic exposure increased viability of SH-SY5Y in a time-dependent manner when compared to controls⁵⁶.

Novák *et al* (2005) showed that MFs have inhibiting influences on the growth of the yeasts *S. cerevisiae*. In the similar study, Strašák *et al* (2005) reported that MFs reduce optical densities of the *S. cerevisiae*⁵⁷. Reviewing the related studies it can be concluded that the MF-induced inhibition can be exerted immediately after the exposure to the yeast culture¹².

Growth Dynamic Assessments

It is important to find out if the inhibitive effects of the MF are bacteriostatic or bactericidal. To answer this question,

Strašák *et al* (2001) ascertained that the slope of the dependence of CFU on the time of the exposure does not equal zero, but it is as to the slope of the control curve. They assumed that cells in the MF do not lose their ability to divide. Death of some bacteria in the culture leads to the reduction of the CFU number. The effect of MFs probably is not bacteriostatic⁴⁵.

Furthermore, Fojt *et al* (2003) surviving growth dynamics observed the reduction of CFU in the sample exposed⁵⁴

These studies suggested that MFs have

no effect on the metabolism of the bacteria. Concluding the previous studies, one can assume that MFs kill those portions of the bacteria with direct exposure.

Dependence of CFU on Magnitude of Magnetic Induction

Magnitude of MF is one of the most important features influencing the bacteria growth. In order to investigate it, bacteria are usually exposed to a MF and the magnitude of the magnetic induction was changed.

Strašák *et al* (2001) and Fojt (2003) demonstrated an exponential decrease of the number of CFU in the exposed culture. The result was again the same as for inhomogeneous MFs⁴⁵.⁵⁴ In this regard, Novak *et al* (2005) exposed the yeast cells culture by MFs, and found that the antibacterial effects were stronger with higher magnetic inductions¹².

In addition, Gomes *et al* (2004) reported the growth effects induced by static and sinusoidal 50 Hz MFs on the haploid yeast strain *S. cerevisiae* WS8105-1C and the experiments were conducted at 0.35 and 2.45 mT (low MF) and the yeasts were exposed to MF for 24 and 72 h in the homogeneous field area. The results demonstrated that static and sinusoidal 50 Hz MF (0.35 and 2.45 mT) did not induce changes in the growth of *S. cerevisiae*²⁴.

Majority of the studies investigating the effects of MF induction on bacterial growth rates, there was a significant relationship between increasing MF induction and decreasing of growth bacteria so that low intensity MFs could not significantly change the growth curve.

According to the critical review of Adair (1997), it is far that <0.05 mT MFs at 50 or 60 Hz can affect other processes than free radical reactions- during their sufficient cage containment time of about 50 ns- suppressing recombination rate by 10 of 40%⁵⁸.

Electromagnetic Fields

This section discusses the effects of EMFs on bacteria. To explain EMFs effect, we can classify EMFs into seven categories: (1) ELF (0-300 Hz), used for biological processes; (2) very low frequency (300-30 KHz); (3) low middle frequency (30 KHz-30 MHz), used for amateur radio and remote controls; (4) ultra high (30-300 MHz), used in radio and TV; (5) super high (300 MHz-30 GHz), used in satellite communication; (6) extremely

high frequency (30-300 GHz), used in radar; (7) infrared (300 GHz-300 THz); and visible light (429-750 THz), used in light spectrum.

In the following, the antimicrobial and antibacterial effects of EMFs in the two main categories are reviewed: High frequency low intensity EMFs and low frequency low intensity EMFs.

High Frequency Low Intensity EMFs

A complex network of sensing and responding to physical and chemical factors is used by living cells, especially by bacteria, to communicate with each other and to survive under different environmental conditions⁵⁹. It was suggested that electromagnetic irradiation (EMI) of extremely high frequency (30-300 GHz) with low intensity at specific resonant frequencies can affect bacteria in the manner of energy transformation into informative signals (70-73 GHz). Accumulating data explain the potential of low intensity coherence EMI of resonant frequencies to cause depressing effects on *E. coli* which is considered the best characterized bacteria and a model organism⁶⁰⁻⁶³.

These effects mainly depend on intensity of irradiation and exposure, the combination of growth and irradiation media, the genetic features of strains, the coordinates of bacterial metabolism and other factors⁶⁴⁻⁶⁶. In addition, these effects can regulate the mutual reaction of organisms against impact of physical and chemical factors^{65, 67}. A mutation in the growth cycle of bacteria is possible due to metabolic processes or mechanical resonance^{68, 69}.

It is known that *E. coli* growth can be decreased at specific frequencies of low-intensity EMI from the ranges of 45-53 GHz and of 70-75 GHz^{63, 67}. One of the possible interaction mechanisms with such EMI is Genome targeting. However, the energy resulting from these frequencies is not sufficient to break a chemical bond in DNA. It is possible that EMI at these levels can create oxygen radicals, or disorder process of DNA-repair processes⁷⁰. The elastic forces in the walls of cell membranes help to weaken oscillatory forces by participating in coherent self-sustained oscillations that lead to possible macromolecular conformational transitions that are fed with metabolic energy⁵⁹. They are driven biologically and need ATP. Thus, the proton F_0F_1 -ATPase, the

main enzymatic complex of the bacterial membrane, can play a key role in membranous mechanisms of EMI action. The latter has been proven with the changes of irradiated bacterial cell sensitivity to N, N'- dicyclohexycarbodiimide (DCCD) - an inhibitor of the F_0F_1 -ATPase^{65, 67, 69, 71}. The change in the oxidation-reduction potential (Eh) of the bacterial surface, which plays an individual role in where bacteria can survive and especially in the regulation of the FOF1-ATPase is another findings⁶⁹. In addition, EMI effects on bacteria can be mediate by water molecules at their own resonant frequencies (41.5, 51.8 and 53 Hz)⁷², and for these frequencies showed dramatic decrease in *E. coli* growth^{64, 65, 67, 69}. The fluctuation of water molecules can alter protein composition and the degree of hydration and other properties of proteins^{60, 62, 64, 66}. The effects on *E. coli* growth and on properties of water molecules have been recently reported for 70.6 and 76 GHz EMI⁶⁴.

Extremely Low Frequency

Various works have been done on the effects of ELF EMFs on biological systems^{73, 74}. The results of ELF-EMF research are contradictory, and little is known about the possible mechanisms of interaction between ELF-EMF and living organisms. Standard methods, such as growth and protein synthesis, were used to survey the effect of ELF-EMFs on bacteria, and specially designed methods were employed to test the influence of ELF-EMFs on bacterial bioluminescence²³.

ELF-EMF has few effects on bacteria. Two considerations should be emphasized: (1) the effect observed may be dependent on the fields which are used; the applied fields should be temporally and spatially coherent and undisturbed by incoherent magnetic or electric noise⁷⁵; ELF-EMFs vary in wave form, frequency and strength; it is possible that a sharp "window" (i.e. a discrete combination of frequency and strength) is necessary to make an effect visible; (2) prokaryotes, which are completely functional, intact organisms, may be more "resistant" than cell cultures and may be able to compensate (atone) for the decrease of an EMF.

Protein Synthesis

Bacteria are known to create stress proteins, e.g. induced by heat. The heat-induced effect was confirmed with *Proteus vulgaris* at 41 °C, which showed a severe change in its protein

pattern tested by IEF. At 37 °C, no influence of the EMF was visible. The combination of heat (41 °C) and EMF led to distinct changes at pH 6. Using *E. coli* and SDS-PAGE no effect could be seen, either at 37 °C or 43 °C. This strain is probably able to sustain even higher temperatures without an alteration in protein pattern, according to the protein synthesis results mentioned above⁷⁶.

Various work reported the influence of ELF-EMF on the protein synthesis of eukaryotic cell cultures^{77,78}. It has been reported that *E. coli* protein synthesis is influenced by sinusoidal 72 Hz MFs in a cell-free system⁷⁹ and by PEMFs in vivo using highly sensitive two-dimensional electrophoresis⁸⁰. No effect was found using 60 Hz sinusoidal MFs⁸¹. Changed in bacterial protein pattern only appeared when heat stress was applied in addition to the MF. Heat seems to play an important role in the combined action with ELF-EMFs. The physiological reaction of eukaryotic cells to heat shock seems to be similar to that induced by ELF-EMF stress^{79,82}. Radical reactions with electron carriers have been reported to be influenced by EMFs⁸³.

Effect of EMI on Enzymatic Activity

The applied EMFs affected the membrane bound enzyme activity but the effect on Triton solubilized disk membranes or on soluble isoforms of adenylate kinase was negligible. Small effects of ELF-EMFs on the activities of soluble enzymes have been reported^{84,85}. These findings indicated that the membrane may play a key role in mediating the effect of the field on the enzymatic activity. Indeed, interesting results involving biological membranes exposed to ELF-EMFs were reported⁸⁶⁻⁸⁹.

Morelli *et al* (2005) found ELF-EMFs of 75Hz with amplitudes above a threshold reduces the enzymatic activities of three membrane-bound enzymes (alkaline phosphates, phosphoglycerate kinase, and acetyl cholinesterase from blood cell or from synaptosomes) by about 54–61%⁹⁰ Falone *et al* (2007) showed the main antioxidant and GSH dependent detoxifying enzymatic activities in control and ELF-EMF-treated neuroblastoma cells. It is clear that ELF exposure significantly increases the activities of glutathione S-transferase and glutathione peroxidase while treatment did not affect superoxide dismutase, catalase and glutathione reductase activities⁵⁶.

Antioxidant Effects

To investigate antioxidant effect of through ELF-EMF treatment, Falone *et al* (2007) tested the possible ELF-EMF-dependent modulation of the cellular vulnerability grade towards a well-characterized pro-oxidant treatment. They found a similar induced mortality of hydrogen peroxide both in cells exposed and in controls. However, long-term ELF-EMF-conditioned neuroblastoma cells showed a significant, increase in ROS generation after H₂O₂ incubation. This rise appeared to be completely reverted by the co-treatment with the well known antioxidant N-acetylcysteine⁵⁶. Therefore, exposure to ELF-EMF may affect the free radicals production or enhance the hydroxyl radicals activity produced by H₂O₂, the main ROS detected by H₂DCFDA.

Growth Curve Assessments

Falone *et al* (2007) have shown that SH-SY5Y growth curve is not affected significantly by ELF-EMF, whereas ELF-EMF exposure increased SH-SY5Y viability in a time-dependent manner⁵⁶.

In many experiments with ELF-EMFs under standard temperature conditions, the growth of *E. coli* K12, the protein synthesis rate of *E. coli* B leu-3 and the luminescence of *Photobacterium phosphorus* and *Photobacterium fischeri* was not significant. Thus, in approximately 10% of the experiments, the significant changes could not be explained as artifacts. EMFs per se were unable to affect significantly intact bacterial cultures. If any effects were detected, they were mostly so tiny that they were shrouded by the biological variance or not reproducible. Growth was reduced by a maximum of 3.8%. Other workers have found similar small effects on the growth of *E. coli*^{91,92}. In other experiments with different strains, such as *Bacillus thuringiensis* and mutants of *E. coli* and *Proteus vulgaris*, no effect on growth was observed²³.

Electrical Fields

In the past, the efficacy of high EFs on living cells has aroused high research interest. As the fields can effectively kill bacteria and yeasts, pulsed EFs (PEFs) of lethal magnitudes have proven useful for food preservation. Investigating experiments mainly carried out on algae, erythrocytes and tissue cells indicates that considerable results are obtained for external EFs in the range of kilo volts^{95,96}.

First in the 1960s, Doevenspeck used and

described EFs to kill microorganisms⁹⁷. Then in 1967 and 1968, Hamilton and Sale analyzed PEFs but not AC EFs on bacterial protoplasts, spheroplasts, and erythrocytes^{98, 99}.

During the PEF process, the biological cells are subjected to an EF with high field strength, allowing plant and animal cells to be opened up. To produce the PEF, both a treatment chamber and a source are required. The treatment chamber contains at least two electrodes, with an insulating region in between them, where the substance is located in there; then the PEF is applied.

The fatal effects of EFs on living cells are probably the outcome of direct interaction between cell membranes and external electrical fields [95, 96, 98-100]. The applied field induces significant potential among biological membranes and so it may cause the loss of the relatively high resistance of the membrane under physiological conditions. This event happens in the cell membranes, induced by enough high induced potentials of short time^{96, 100}. Approximately all cells have pores which control the flow of wastes and nutrients into and out of the cell.

The process of micro-organisms inactivation which is induced by EF has multiple steps. Saulis proposed that the effect of PEF treatment upon microorganisms during food processing consists of four main stages: (1) increment in the transmembrane potential due to charging the cell plasma membrane by the external EF applied, (2) pore initiation stage, (3) measurement of the pore population during an electric treatment (4) post-treatment stage (pore resealing, cell death)¹⁰¹.

The main efficacies of PEF on microbial cells depend on the amplitude of pulse, size of the cell and include structural fatigue due to induced membrane potential and mechanical stress¹⁰² with duration from nanoseconds to milliseconds¹⁰¹.

When an EF exposes the cell, the free charges which are created on the membrane surfaces are moved to one another because of the difference in the signs (- and +) which causes a compression.

Transmembrane potential is induced by accumulation of positive and negative charges in cell membranes. Potential induced by field application is superimposed onto the initial transmembrane potential. Potential which induced

on the cell membrane is important for investigating the effects of the EF on cells and can be calculated analytically or numerically¹⁰³. The electrostatic attraction between the two sides of the membrane may increase with thinning membrane. Local membrane breakdown with pore formation occurs for a given value of the applied field. High transmembrane potential applies pressure on the membrane of cell; then this pressure reduces thickness of membrane and eventually causes pore formation. Once potential is approximately 1 V, Cell lysis with loss of membrane integrity occurs⁹⁸. Following exposure to PEF treatment, the microorganism dies¹⁰⁴.

Usually, the intensity of the EFs is on the order of 20 kV/cm and the durations are 1 to 300 μ s. usually, the number of pulses is on the order of 10. This phenomenon occurs at low or moderate temperatures without causing significant sensorial quality changes

Type of Microorganisms

Barsotti and Cheftel demonstrated that the efficiency of microbial inactivation depends first on the type of microorganism¹⁰⁵. Some investigators have found that Gram-positive bacteria are more resistant to EF compared with Gram-negative, and yeasts exhibit more sensitivity to EFs than vegetative bacteria¹⁰⁶⁻¹⁰⁸.

Cell Size and Shape

The size and shape of a microorganism play a significant role in its inactivation during exposed cells by EF^{109, 110}. The cells with smaller diameters are killed at higher electric direct field than the cells with larger diameter^{93, 111, 112}, they are less resistant to alternating current, compared with larger cells. The effects of the cell size and cell shape on the fatal effect of EF have been related to the transmembrane potential generated by strengths of external EF. Qin *et al*, Hülshager *et al* and Stoica *et al* found that when the cell volume increases, a decrease in critical breakdown potential occurs^{109, 112, 113}.

Electric Wave

The most important parameter affecting the performance of microbial inactivation by PEF is EF intensity¹¹⁴.

The process of PEF involves the application of high voltage pulses, usually of 20-80 kV/cm for short periods of time (less than 1

second)⁹⁴. When the applied EF becomes more than a critical value for a certain period of time, the transmembrane potential is induced and then leads to cells dying. From the EF strength and the period of exposure time, some other variables such as pulse characteristics can also influence the inactivation ratio and reaction kinetics in PEF treatment¹⁰⁷. Usually, the square wave and exponential decay pulses are used for PEF process^{102,115}.

Medium Conductivity

The conductivity of medium influences considerably the action of the EF which transits through that medium¹¹⁶⁻¹¹⁸ and in this state there are living cells. The medium conductivity is an important factor that affects the biological properties⁹⁴. The electric medium conductivity is an important parameter in EF process¹¹⁵. The correlation between inactivation of microorganism and medium electrical conductivity has been studied by some authors^{112, 114, 119-123}. Some investigators discuss that the process of PEF treatment is more efficient in medium with lower conductivity because of a larger difference on the concentration of ionic between the suspension and the cell cytoplasm^{115, 120}. The large ionic slope facilitates an increase by ionic substances among the cell membrane, which weakens the structure of membrane and makes it more sensitive to the PEF^{94, 115}. Therefore, more researches argue that the inactivation of microorganism increases with reducing the medium conductivity¹²⁴. Other investigators have demonstrated that by decreasing the medium conductivity it is feasible to increment the inactivation level of yeast strains, such as *S. cerevisiae* or other microorganisms^{93,123}.

Ionic Strength and Medium PH

The microbial inactivation by PEF technology is extremely influenced by strength of ionic and medium pH¹⁰⁷. When the medium has a low ionic strength, the inactivation ratio is usually increased^{94, 107, 112, 120, 121}. Vega-Mercado *et al* consider that the ionic strength and PH disturb the homeostasis of the microorganisms leading to an increase of the inactivation ratio¹²¹. Increasing the ionic force leads to an increment in the electron mobility through solution and reduction in the microorganism inactivation by PEF treatment. Tsong reported that the reduced inactivation rate

in high ionic force solutions can be described by the cell membranes stability when they are exposed to a medium which includes several ions¹²⁴. The ions which dissolved in the treated medium such as Ca²⁺, Na⁺, K⁺, Mg²⁺ have been found to reduce the effects on microbial inactivation with EF¹¹². Bruhn *et al* find out that the presence of ions in a medium looks to be necessary to increase the transmembrane potential¹²⁵. Some researchers have demonstrated that in acidic medium, microorganisms were more sensitive to the PEF^{51,98,121}. Other investigators have showed that resistance of microbe was lower at neutral pH¹²⁶⁻¹²⁸ and with no influence on microbial EF inactivation^{126,129}. These differences have not been definite yet, but researchers could be correlated with the increasing number of pulses and EF power applied at the medium which has lower pH, the microorganism's type¹³⁰ and a change in the cell ability to maintain a transmembrane pH gradient because of membrane electroporation¹³⁰. The medium pH plays a significant role in microbial inactivation when EF is combined with organic acids treatment having antimicrobial effect¹³¹. The strong synergic inactivation by composition of organic acids and PEF treatment at lower pH (e.g. 3.4) indicated that entry of undissociated acids into microbiological cells was enhanced³³.

CONCLUSION

The present study has reviewed the most current techniques of EMFs in antimicrobial studies and mechanisms of actions of these methods. EFs, MFs and PEMFs show the promising antibacterial effects. These techniques in appropriate parameters can be used for some bacterial and microbial pathogens as alternative and adjunctive treatment options. For establishing new EMFs based techniques for antimicrobial and antibacterial purposes further control studies should be performed.

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