Bio-impedance detector for Staphylococcus aureus exposed to magnetic fields

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Abstract. Rapid detection of viability and growth of pathogenic microorganisms is very important in many applications such as food and drug production, health care, and national defense. Measurements on the electrical characteristics of cells have been used successfully in the past to detect many different physiological events. The effect of electromagnetic fields on the growth of bacteria (Staphylococcus aureus) was studied with the bio-impedance technique. The growth situations of bacteria in the absence and presence of different intensities of static and alternative magnetic fields were examined and analyzed. The results show that the impedance of bacteria fell in the presence of DC magnetic fields. In contrast the impedance increased when the bacteria were exposed to AC magnetic fields. Based on these results the bacterial growth indicated by the change in the impedance is inhibited under DC magnetic fields and enhanced under AC fields.

1. Introduction

Many biological parameters and processes can be sensed and monitored using their impedance as marker with the advantage that it is a non-invasive-and a relatively cheap technique [1 and 2]. Cell growth, cell activity, changes in cell composition, shapes and cell location are only some examples of processes which can be detected by the impedance method. The electrical impedance of a biological sample reflects actual physical properties of the tissue. Electrical bio-impedance can be used to assess the properties of biological materials involved in processes such as cancer development [3]. Because the cells of healthy tissues and cancer are different in shape, size and orientation, abnormal cells can be detected using their impedance as a marker. Impedance Z can be represented by the ratio between the voltage and current. Thus, any alteration in the components of the current will alter the voltage current relationship and result in an impedance change. The impedance measured depends on a number of variables, such as adhesion tightness, cell type, and surface area of the electrode, frequency, and confluence of cells [4]. The exposure of living tissue to various types of magnetic fields is a commonly encountered event: extremely low frequency from power lines, high frequency electromagnetic fields (EMF) from cellular phones, and computers [5]. Since this is a task of medical and technological importance, a number of attempts have been given to clarify the effects of magnetic fields on biological cells. [6]. The resurgence of interest in the interactions between electromagnetic fields and biological systems has mainly focused on AC (time-varying) fields. However, there have been studies showing that DC (static) magnetic fields can also interact with living systems at various levels. Nosocomial infections remain a serious problem in medical institutions. Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen and continues to trigger outbreaks of infection. Efforts to control such outbreaks are of crucial importance not only for the sake of protecting patients but also in preventing the tremendous costs that arise within the context of an epidemic [7]. Experimental investigations on how static magnetic fields and low-frequency magnetic fields can affect the growth dynamics of bacteria Staphylococcus aureus have been done by many
other scientists [8 and 9]. The objective of this study is to test the effect of DC and AC magnetic fields on the Staphylococcus aureus growth recorded by the bio-impedance technique.

2. Theoretical aspects
Impedance can be defined as the resistance to the flow of an alternating current as the current passes through a conducting material. When two metal electrodes are immersed in a conductive medium the test system behaves as a resistor and a capacitor in series. When the system is treated as a series combination of the resistance and the capacitance an applied sinusoidal potential will produce a resultant current which depends on the impedance of the system [10]. The impedance in turn is a function of the resistance, capacitance and applied frequency. Microbial metabolism usually results in an increase in both conductance and capacitance causing a decrease in impedance and a consequent increase in admittance. The change in conductance occur in culture medium due to metabolism of uncharged or weakly charged substrates which are converted to highly charged end products, e.g. proteins to amino acids. Capacitance is related to the behavior of ions at the surface of the electrodes and can be monitored separately from conductance [11].

3. Materials and methods

3.1. Impedance System Fabrication
The impedance system was fabricated in a plastic tube of a diameter of 0.5 cm and a length of 4 cm with four silver electrodes (Ag 92.5gm to Cu 7.5gm) fixed inside the tube. These electrodes had a diameter of 0.5 cm and a thickness of 0.2 cm and they were arranged at equal distances of 0.5 cm apart as shown in figure (1).

3.2. Bacteria Culturing
Specimens were collected from patients in Azadi hospital in Duhok city-Kurdistan region, Northern Iraq. The isolates were recovered from nasal swabs of patients. For isolating the bacteria (Staphylococcus aureus), samples were cultured on the blood agar (HI-MEDIA) in an aerobic atmosphere. After incubation at 37°C for 24 hours, colonies of Staphylococcus aureus on blood agar appeared yellowish and usually surrounded by a zone of haemolysis. Then the sample was sub cultured on mannitol salt agar (HI-MEDIA) and incubated at 37°C for 24 hours. Colonies characterization was generally yellow surrounded by a yellow zone caused by the fermentation of mannitol. Standard microbiological methods for the identification of Staphylococcus aureus were followed including Gram staining, biochemical tests (catalase, coagulase and DNase) and the bacteria were identified further by API staph method (Biomerieux- France).

3.3. Setup of Magnetic Fields
A pair of conducting circular coils, (Helmholtz Coils with a radius of 14 cm) each coil having 320 turns, carrying different values of current I and with the coils separated by a distance equal to half of the radius (7 cm) of the circular loops, produces a homogeneous magnetic field B in the mid-plane between the two circular coils. The strength of the magnetic field was measured at a point in space called the field point. This point was located in the mid-plane between the two coils. Direct (DC) and alternative (AC) currents (50 Hz) were applied separately in simple series circuits as shown in figure (1).
3.4. Impedance Measurement
The microbial impedance changes were measured with a pair of electrodes placed in a growth medium or reacting solution. This measurement was taken over a frequency range of 100 Hz to 20 KHz from the tube filled with bacterial broth. The tube was incubated at 37°C for the duration of the experiment.

The voltage of the applied sinusoidal signal was about 1V\textit{rms}. The impedance of the bacterial samples was determined by the ratio of the measured potential drop $\Delta V$ between the two inner electrodes and the electrical current $\Delta I$ which depends on the measurement frequency and is supplied at the two outer electrodes. In addition to the control samples (not exposed to magnetic field), the impedance of dead bacteria was determined similarly by heating up a test sample to 75 °C and exposing the sample to different magnetic fields. Another test was carried out to measure the impedance of the control sample as a function of time.

3.5. Statistical Analysis
The statistical analysis \textit{ANOVA single factor} was used to perform a simple analysis of the variance of the data. The values described with (p < 0.05) were considered as significant (Excel 2003).

4. Results
4.1. Impedance Measurements
The impedance changes associated with the growth of Staphylococcus aureus were calculated as a function of frequency between a pair of Helmholtz Coils. At the beginning two preliminary experiments were done. In the first experiment the impedance of the heated dead Staphylococcus aureus was acquired as a function of the frequency. The result is shown in figure (2).
Figure 2. frequency responses for the impedance of control and dead Staphylococcus aureus.

Figure 3. the significant changes of impedance values as a function of frequency for the control and dead Staphylococcus aureus.

Figure 4. the significant changes of impedance values as a function of frequency for the control and two days growth of Staphylococcus aureus.

In the second experiment the impedance of the Staphylococcus aureus grown for two days was measured as a function of the frequency. The results are shown in figure (4) and indicate that variations of impedance caused by the bacterial growth could be detected by the bio-impedance system. The changes ($P < 0.05$) were significant in comparison to the control sample which clearly appears in figure (3). These results were in good agreement with data reported in [12, 13 and 14].

4.2. DC Magnetic Fields
To test bacterial growth exposed to static magnetic fields using the bio-impedance system, the impedance values of magnetized (test) and un-magnetized (control) samples of bacteria were measured (table 1). The test samples were exposed to the magnetic field densities of 0.1, 0.2, and 0.3
mT at different frequencies ranging from 100 Hz to 20 KHz. The effect of DC magnetic fields on impedance variations is shown in figure (5). The impedance variation was significant (P< 0.05) only at low frequencies (50-100 Hz) as shown in figure (6).

![Figure 5](image-url)  
**Figure 5.** Frequency responses for the impedance of Staphylococcus aureus exposed to different DC magnetic fields.

![Figure 6](image-url)  
**Figure 6.** The significant changes of impedance values as a function of frequency (100Hz and 20 KHz) for Staphylococcus aureus exposed to different DC magnetic fields.

### 4.3. AC Magnetic Fields

To test bacterial growth exposed to alternative magnetic fields using the bio-impedance system, the impedance values of magnetized (test) and un-magnetized (control) samples of bacteria were measured (table 2). The test samples were exposed to the magnetic field densities of 0.11, 0.21, 0.5, and 0.7 mT at different frequencies ranging from 100 Hz to 20 KHz. The effect of AC magnetic fields on impedance variations is shown in figure (7). The significant changes of the control and magnetized samples are also summarized in fig. 8. The most important contribution to the impedance change at low frequencies is related to the capacitance of the medium, which is dependent on the frequency.
Table 1: Impedance response vs. frequency for *Staphylococcus aureus* exposed to different DC magnetic fields.

| Frequency /KHz | Impedance (Ohm) |
|----------------|-----------------|
|                | Control 0 mT    | DC Magnetic Field 0.1 mT | DC Magnetic Field 0.2 mT | DC Magnetic Field 0.3 mT |
| 0.05           | 118.42          | 125.00                    | 126.76                     | 128.57                     |
| 0.10           | 117.58          | 118.18                    | 121.18                     | 121.43                     |
| 0.40           | 112.90          | 113.33                    | 113.56                     | 113.56                     |
| 0.50           | 111.45          | 111.90                    | 112.10                     | 113.01                     |
| 0.60           | 111.03          | 111.45                    | 111.63                     | 111.72                     |
| 0.80           | 109.72          | 109.29                    | 110.22                     | 110.29                     |
| 0.90           | 108.84          | 109.09                    | 109.22                     | 109.29                     |
| 1.00           | 108.00          | 108.22                    | 109.09                     | 109.15                     |
| 2.00           | 103.05          | 103.13                    | 103.16                     | 103.18                     |
| 3.00           | 98.25           | 97.62                     | 97.59                      | 98.18                      |
| 4.00           | 93.71           | 93.60                     | 93.53                      | 94.08                      |
| 5.00           | 90.40           | 90.29                     | 90.17                      | 90.70                      |
| 6.00           | 88.20           | 87.57                     | 88.00                      | 87.93                      |
| 7.00           | 85.56           | 85.39                     | 85.80                      | 86.29                      |
| 8.00           | 84.44           | 83.80                     | 84.18                      | 84.18                      |
| 9.00           | 82.87           | 82.68                     | 82.58                      | 82.58                      |
| 10.00          | 81.77           | 81.11                     | 82.02                      | 81.46                      |
| 20.00          | 73.03           | 71.91                     | 72.32                      | 72.32                      |
Table 2 impedance response vs. frequency for staphylococcus aureus exposed to different AC magnetic fields

| Frequency /KHz | Control 0 mT | AC Magnetic Field 0.11 mT | AC Magnetic Field 0.21 mT | AC Magnetic Field 0.5 mT | AC Magnetic Field 0.7 mT |
|----------------|--------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 0.10           | 131.33       | 126.19                    | 127.38                    | 125.58                    | 125.29                    |
| 0.40           | 125.00       | 122.03                    | 121.01                    | 120.66                    | 121.67                    |
| 0.50           | 123.77       | 120.97                    | 120.00                    | 120.63                    | 119.84                    |
| 0.60           | 124.60       | 120.16                    | 119.23                    | 119.08                    | 119.23                    |
| 0.80           | 122.39       | 119.85                    | 119.12                    | 118.98                    | 119.12                    |
| 0.90           | 121.17       | 118.71                    | 118.71                    | 117.86                    | 117.99                    |
| 1.00           | 120.86       | 118.31                    | 117.61                    | 117.61                    | 116.90                    |
| 2.00           | 117.11       | 114.94                    | 113.64                    | 112.99                    | 112.99                    |
| 3.00           | 112.58       | 109.94                    | 109.32                    | 108.70                    | 109.38                    |
| 4.00           | 109.26       | 107.32                    | 106.71                    | 106.75                    | 106.75                    |
| 5.00           | 107.36       | 104.82                    | 104.22                    | 103.61                    | 103.61                    |
| 6.00           | 104.85       | 102.99                    | 102.40                    | 102.40                    | 102.40                    |
| 7.00           | 103.01       | 101.19                    | 101.19                    | 100.60                    | 100.60                    |
| 8.00           | 102.41       | 100.60                    | 99.41                     | 99.41                     | 99.41                     |
| 9.00           | 100.60       | 98.82                     | 98.22                     | 98.22                     | 98.22                     |
| 10.00          | 99.40        | 98.22                     | 97.06                     | 97.06                     | 97.06                     |
| 20.00          | 90.91        | 89.76                     | 89.22                     | 89.22                     | 88.69                     |

5. Discussion
A simple and rapid impedance method was proposed to detect bacterial growth exposed to DC and AC magnetic fields by making use of the properties of bacterial cell suspensions and by using a four electrodes system. The impedance values of the bacterial growth were measured to find their dependency on the magnetic fields applied to control the bacterial growth.

The significant variations of impedance shown in figures (3 and 5) indicate that the change in impedance figures (2 and 4) is suitable as a parameter indicating bacterial growth. Absolute values of the changes in impedance could be obtained as a consequence of bacterial growth. These changes in time are explained by the metabolic products created during the growth of microorganisms. The metabolic products modify the composition of the medium, thus changing the ionic content, which in turn produces a change in the conductivity of the culture media [15, 12, 16 and 17].

The results reinforce the concepts that the change in impedance is the consequence of bacterial growth. This significant relationship between growth of bacteria and its electrical properties (Impedance change) has been harnessed to clarify the effects of magnetic fields on biological cells. These effects on the bacteria have been recorded through impedance measuring techniques. In case of applying DC magnetic fields, the impedance values of the exposed bacterial sample increase which are significantly higher than that of the control sample as shown in figures (5 and 6). This indicates that the bacterial growth was inhibited when exposed to DC fields. In other words the growth of these bacteria was negatively affected by increasing the static magnetic field which aligns rather well with results reported in [8, 18, 19, 20, and 21]. Invariably the growth of the culture was associated with a topping up in impedance of about 5.2, 6.6 and 7.9 % for 0.1, 0.2, and 0.3 mT magnetic fields respectively comparing to the impedance of the control cells. This agrees with the observation that the bacterial growth decreases with increasing DC magnetic fields intensity [8, 22].
In contrast, the drop in the impedance values of bacteria exposed to AC magnetic fields figures (7 and 8) indicates that bacterial growth was enhanced. This means that when the bacteria were exposed to an alternating field, a positive effect was observed on the rate of growth of the colonies [8, 19, 23, 24, and 25]. Table 2 shows that the growth of the bacteria was associated with a drop in impedance of about 3.9, 3, 4.3 and 4.6 %, for 0.11, 0.21, 0.5, and 0.7 mT AC fields respectively in comparison to the impedance of the control cells.

Opposite to the observations when DC fields were applied, these values suggest that there is no substantial contribution of the increasing AC fields to the impedance change. This means that the bacterial growth is independent of AC field intensities since approximately a similar relative impedance drop was produced for all field intensities examined [26]. It is clear that there is no linear decrease of impedance values depending on increase of magnetic fields. The major contribution to the impedance change for the bacteria cells can be viewed at low fields.

6. Conclusion
It can be concluded that the impedance variation is a good indicator of bacterial growth in colonies of bacteria exposed to magnetic fields. The changes in the medium during bacterial growth subject to magnetic fields can be detected by measuring impedance only at low frequencies. In DC fields the impedance values increase which reflects a decline in bacterial growth. On the other hand, impedance values drop in AC fields which reflect enhancing bacterial growth.

7. Reference
[1] Alberto Yúfera, Daniel Cañete and Paula Daza 2011 [A Microelectrode-Cell Sensor Model for Real Time Monitoring] SENSORDEVICES: The Second International Conference on Sensor Device Technologies and Applications
[2] Meas. Sci. Technol, Sanchez B, Vandersteen G, Bragos R and Schoukens J 2011[Optimal multisine excitation design for broadband electrical impedance spectroscopy]. Meas. Sci. Technol 22 115601
[3] Rohit Dua, Daryl Beetner G, William Stoecker V, and Donald Wunsch C 2044 [Detection of Basal Cell Carcinoma Using Electrical Impedance and Neural Networks] IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING, 51 1
[4] Wiertz R.W. F., Rutten W. L. C. , and Marani E 2010 [Impedance Sensing for Monitoring Neuronal Coverage and Comparison With Microscopy] IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING, 57 10
[5] Ikehata, M., Koana T, Suzuki Y, Shimizu H and Nakagawa M, 1999 [Mutagenicity and co-mutagenicity of static magnetic fields detected by bacterial mutation assay]. Mutation Research 427, 147-156
[6] Nakasono S. and Saiki H 2000 [Effect of ELF magnetic fields on protein synthesis in Escherichia coli K12]. Radiation Research 154 208-216
[7] Wicdlhaus, T, Hunfeld, K, Bodinghaus, B, Kraiczzy P, Schafer V and Brade V 2001 [Rapid molecular typing of MRSA by PCR-RFLP] Infection Control and Hospital Epidemiology 22 294-298
[8] Perez Medina 2010 [EFFECT OF WEAK MAGNETIC FIELDS ON BACTERIUM STAPHYLOCOCCUS] AUREUSInicio 11
[9] Masahiro Kohno, Muneyo Yamazaki, Isao Kimura and Moriyasu Wada 2000 [Effect of static magnetic fields on bacteria: Streptococcus mutans, Staphylococcus aureus, and Escherichia coli] Pathophysiology 7 2 143-148
[10] Yang L, Ruan C and Li Y 2003 [Detection of viable Salmonella typhimurium by impedance measurement of electrode capacitance and medium resistance] Biosens Bioelectron 19 5 495-502
[11] Bolton F J In 1991[Conductance and Impedance Methods for detecting Pathogens] Rapid Methods and Automation in Microbiology and Immunology 176-181
[12] Nardo Ramírez, Angel Regueiro, Olimpia Arias and Rolando Contreras 2009 [Electrochemical impedance spectroscopy: An effective tool for a fast microbiological diagnosis]. Biotecnología Aplicada 26 72-78

[13] STRATEN, VAN R and SONDERKAMP H J 1978 [Impedance as a parameter for bacterial growth in medical microbiology]. Antonie van Leeuwenhoek 44

[14] Tao Zhu, Zhenhua Pei, Jianyong Huang,ac Chunyang Xiong, Shenggen Shib and Jing Fanga 2010 [Detection of bacterial cells by impedance spectra via fluidic electrodes in a microfluidic device]. Lab Chip 10, 1557–1560

[15] Owicki J and Parez J 1992 [Biosensors based on the energy metabolism of living cells: the physical chemistry and cell biology of extracellular acidification]. Biosens Bioelectron 7 257-72.

[16] James B Allison, John A Anderson and William H Cole 1938 [The Method of Electrical Conductivity in Studies on Bacterial Metabolism]. J. Bacteriol 36 6 571

[17] Lasik M and Nowak J 2010 [Electrical impedance for bacterial metabolic activity screening-evaluation of single and mixed bacterial consortia for wastewater biodegradation]. International Food Research Journal 17 591-599

[18] Wenjin Ji, Huimin Huang, Aihua Deng and Chunyang Pan 2009 [Effects of static magnetic fields on Escherichia coli]. Micron 40 8 894-898

[19] Piatti E, Albertini MC, Baffone W, Fraternale D, Citterio B, Picentini MP, Dacha M, Vetrano F and Accorsi A 2002 [Antibacterial effect of a magnetic field on Serratia marcescens and related virulence to Hordeum vulgare and Rubus fruticosus callus cells]. Mol Biol 132 2 359-65

[20] Zhang S, ei W, Zhang J, Mao Y and Liu S 2002 [Effect of static magnetic field on growth of Escherichia coli and relative response model of series piezoelectric quartz crystal]. Analyst. Mar 127 3 373-7

[21] Kohno M, Yamazaki M, Kimura I and Wada M. 2000 [Effect of static magnetic fields on bacteria: Streptococcus mutans, Staphylococcus aureus, and Escherichia coli]. Pathophysiology 7 2 143-148

[22] Molouk Mohammed Khazan Alkhazan1 and Amna Ali Nasser Saddiq 2010 [The effect of magnetic field on the physical, chemical and microbiological properties of the lake water in Saudi Arabia]. Journal of Evolutionary Biology Research. 2 1 7-14

[23] Luiz F.C. NASCIMENTO, Galdenoro BOTURA Jr. and Rogério P. MOTA 2003 [GLUCOSE CONSUME AND GROWTH OF E. coli UNDER ELECTROMAGNETIC FIELD]. Rev. Inst. Med. trop. S. Paulo 45 2 65-67

[24] Andrea Amaroli, Francesca Trielli,1 Bruno Bianco, Stefano Giordano,2Elsa Moggia, and Maria Umberta Delmonte Corradol 2006 [Effects of a 50 Hz Magnetic Field on Dictyosteliumdiscoideum (Protista)]. Bioelectromagnetics 27 528-534

[25] Bersani, B Del Re · F, Agostini C, Mesirca P and Giorgi G 2004 [Various effects on transposition activity and survival of Escherichia coli cells due to different ELF-MF signals]. Radiat Environ Biophys 43:265-270

[26] Luigina Cellini, I Rossella Grande,1 Emanuela Di Campli, Soraya Di Bartolomeo,1Mara Di Giulio,1 Iole Robullo, Oriana Trubiani, and Maria A. Mariggio 2008 [Bacterial Response to the Exposure of 50 Hz Electromagnetic Fields]. Bioelectromagnetics 29 302-311