Effects of 900-MHz Radio Frequencies on the Chemotaxis of Human Neutrophils *in Vitro*

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***Abstract*—The effects of radio frequency (RF) fields on the ability of human neutrophils to follow concentration gradients of Cyclic Adenosine 3', 5'-Monophosphate (C-AMP) are reported. Blood from healthy adult donors was exposed *in vitro* to different temperatures and 900-MHz RF field at approximately 0.4 V/m. It was observed that the neutrophils' speed increased with increasing temperatures from 35 °C to 40 °C where it peaked and then decreased above 40 °C without RF exposure. When 900-MHz RF field was applied, the speed increased above the value observed at the same temperature, and the maximum speed exceeded that measured value at any temperature by approximately 50%. The calculated temperature change resulting from the RF exposure was less than one microdegree. The direction of motion changed from along the concentration gradient and the electrical field lines to motion at right angles to the concentration gradient and the electric field. The average time for the neutrophils to respond to the effect of RF radiation was about 2.5 min.**

***Index Terms*—Neutrophils, radio frequency (RF), speed, temperature.**

I. Introduction

THE PUBLIC concern about the possible health effect of radiation from cell phones has increased over the past few years with increased use of cell phones. The possible interaction between the radio frequency (RF) electromagnetic radiation and its biological effects on human tissues particularly on the brain, cancer, and the human immune system is a focus of some of these concerns [1], [2]. Previous work with lasers and the development of the strip diffusion technique for studying chemotaxis [3] lead us to consider this approach as a way of studying the possible effects of radio frequency electromagnetic fields on neutrophils as a component of the body's immune system. These are the easiest to observe under a microscope and comprise 50%–70% of all leukocytes found in blood. There are a number of factors that affect the motion of neutrophils. We are able to control some of these factors such as temperature and the chemo-attractant concentration gradients. However, there are other factors that we did not control in these preliminary experiments where our primary objective was to see if the applied fields had any effects at all. For example, the cell's ability to move depends on the type of leukocyte and its age, the amount of anticoagulant used, and other unknown variables in the condition of the human donors. Therefore, we are not surprised that we did not get the same results in every experiment. We found changes in the speed and direction of motion for the neutrophils in approximately 75% of the experiments that showed a well-defined chemotatic response to C-AMP.

Manuscript received October 27, 2006; revised June 3, 2007. *Asterisk indicates corresponding author.*

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Digital Object Identifier 10.1109/TBME.2007.912636

II. Materials and Methods

Blood was drawn from eight different students and used to make one or two slides on a given day over a period of four years. More than 125 slides have been tested and used for a verity of experiments including exposures to low-frequency electric and magnetic fields as well as those being reported in this paper. Typically one or two cells were used on a given slide as a typical experiment would take an hour or two. Venous blood from a donor's arm was drawn using a 22G11/2 Precision Glide blood collection needle, or by using the finger-stick method, into 4.5-ml vacutainer tubes lined with 0.5 ml of sodium citrate. Once the blood was collected, the tubes were slowly turned upside down several times to effectively mix the anticoagulant with the blood. The tubes were then centrifuged for 3 min at 150 G using a SERA-FUGE II centrifuge machine. Separate layers were formed of red blood cells, leukocytes (white blood cells) including neutrophils, platelets, and serum. The mixture of neutrophils and platelets was concentrated at the boundary between the plasma and the red blood cells. A small sample containing neutrophils, platelets, and few red cells was drawn from the boundary layer using a micropipette. To create a concentration gradient of the chemo-attractant a stripe source diffusion technique [3] was used. Between 1.3 to 3.3 mg of C-AMP (Cyclic Adenosine3', 5'-Monophosphate) was dissolved in between 0.05 to 0.1 ml of serum to obtain a known concentration of the chemo-attractant. Then a needle was used to make a stripe ($\approx$ 1 mm wide) of the solution on the slide. Once the solution stripe was completely dry, a micropipette was used to extract a small sample of neutrophils and they were placed about 0.5 cm from the left side of the microscope slide. The drop of fluid containing neutrophils was covered with a cover slip to create a layer of about 15 μm thick. Then the cover slip was slid to the point such that C-AMP strip was covered along the cover slip's one side. The chemo-attractant diffused and created a concentration and concentration gradient that were numerically calculated as functions of time and position by using the equations developed in [3] and [4]. The cover slip was sealed with Vaseline around the edges to reduce the effects of pressure, temperature, and vibration on the movement of the leukocyte cells and to prevent the sample from drying out. We observed that the samples remained moist for up to 24 h. The slide was then placed on the microscope stage that had been heated to the desired temperature. The heater had no measurable effect on the RF field pattern and the 60-Hz fields were much less than 1 μT.

A 900-MHz signal generator at a measured output power of 20 mW was used to drive a 50Ù coaxial cable that was connected to the input to the microstrip line by two short lead wires. Using transmission line theory, the measured impedance characteristics of the line and its length total RF power into the fluid system was calculated to be approximately 88.4 μW, with an electric field of 0.392 V/M in the fluid. The temperature rise resulting from the RF power was calculated by solving boundary value problem for a finite region of the fluid confined between the cover slip and the microscope slide. The estimated temperature rise in the solution under the cover slip due to RF exposure was less than 10^{-6} °C and the thermal time constant was approximately 250 μs. It was measured to be less than the 0.1 °C resolution of our measuring equipment.

The slide was placed on the heated stage for about 15 min, thus allowing it to reach steady state. A neutrophil was identified by finding a cell that was 1.5 to 2.5 times the size of a red blood cell, changing shape, and moving. After a neutrophil was found meeting the above requirements, its movements were tracked on the computer monitor with the help of a tracking system as function of time and position. Control data were determined by running a series of experiments, at a constant

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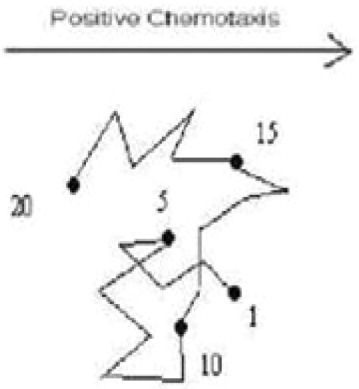


Fig. 1. Random movement of a neutrophil.

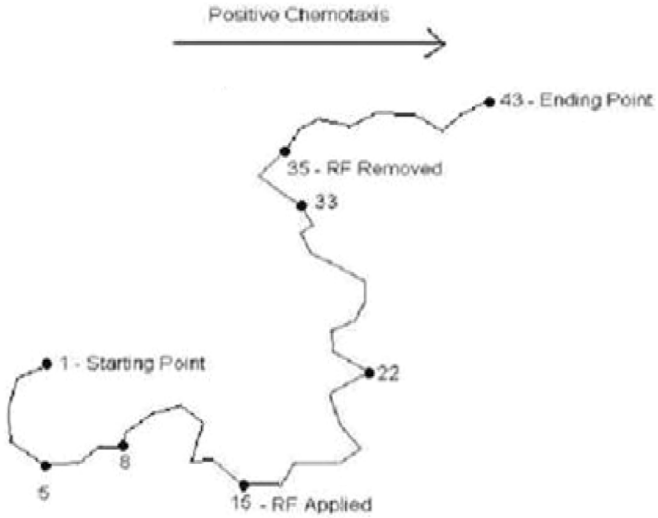


Fig. 2. Typical movement of a neutrophil with RF excitation.

strip concentration of C-AMP and incrementing the temperature by one degree from 22 °C to 43 °C.

III. Results

An example of random motion for a neutrophil moving prior to its sensing the concentration gradient of C-AMP is shown in Fig. 1. The experimental results are based on the observations of the motion of 60 active neutrophils, of which 47 were measured both with and without exposure to the RF fields. These neutrophils were obtained from the fresh blood of eight students. For this set of experiments, the initial concentration of C-AMP was kept constant and the temperature was varied from 22 °C to 43 °C both with and without the application of the RF. For the exposed cells, the motion of the cells was observed for about 15 min before applying the RF radiation and for about 15 min after application of RF radiation. A typical tracking of neutrophil on the calibrated screen is shown in Fig. 2. It is to be noted that in the absence of RF field the average velocity is reasonably independent of the concentration gradient but is a function of the concentration [3]. The following results were established after analyzing the collected data.

1) The neutrophil's speed increased by raising the temperature between 35 °–40 °C and decreased above 40 °C. See Fig. 3.
2) Under the RF radiation, the neutrophil's speed increased by about 50% above the speeds at the same temperatures without the RF. See Fig. 3. Using the data shown in the temperature range from approximately 36 °C to 41 °C the average velocity for 60 cells without RF exposure was $\langle v \rangle = 3.13 \pm 1.08\ \mu$/min (mean and standard deviation). With the exposure of approximately 0.4 V/m at 900 MHz, the average velocity for 47 cells was $\langle v \rangle = 6.46 \pm 0.88\ \mu$/min. The estimated probability that this shift in the average speed occurs by chance gives value for $p \approx 10^{-4}$. This p value was estimated assuming that the distributions at a given temperature both with and without the RF field were Gaussian and that the p value is given by the complimentary error function.
3) Upon RF exposure, approximately 72% (13 out of 18) of the neutrophils moved perpendicular to the direction along the C-AMP gradient and to the applied electrical fields at all temperatures between 35 °C and 42 °C. Without RF these cells moved toward the C-AMP stripe.
4) Significant change in neutrophil behavior, which includes shrinking, expanding, and rolling, was observed under RF.
5) The average time for the neutrophil to respond to the effects of RF was about 2.5 min at this field strength.
6) Neutrophils from old blood (3–4 days) exhibited zero activity. One and two day's old blood (stored in refrigerator) provided neutrophils that exhibited active behavior but remained less active than the fresh blood.
7) No effects from RF were observed on the platelets or red blood cells.

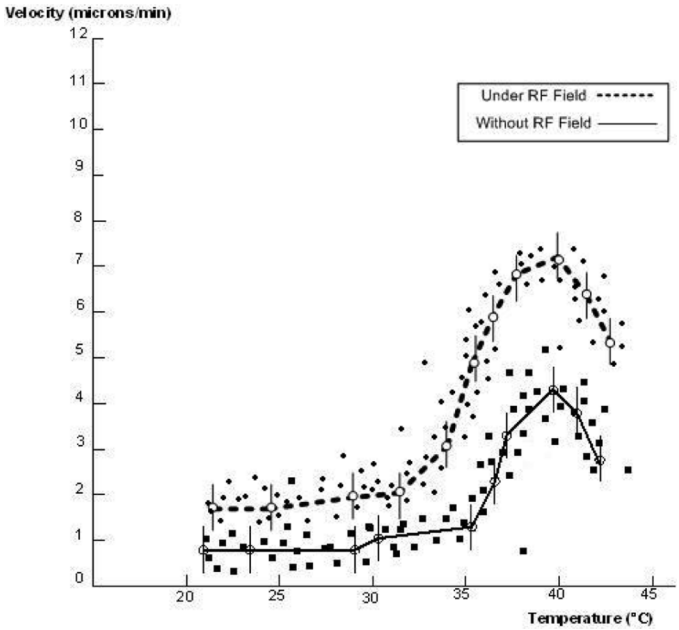


Fig. 3. Cell Velocity as a function of temperature with and without exposure to RF fields at 900 MHz and approximately 0.4 V/m.

IV. Discussion and Future Work

These results are preliminary. There is much more work to be done to determine the effects of variations in intensity, length of exposure time, and frequency to understand the reasons for the observed response of neutrophils to RF fields. Both the measurements and the calculated temperature increase for the application of the RF fields are so small that they seem to be an unlikely mechanism for the observed changes in speed. There is also a need to understand the effects of other cells on the movement. Exposures to ELF have been shown to stimulate the release interleukins including IL-1, IL-6 and other cytokines [5]. IL-1 is known to stimulate the activity of neutrophils, and there may well

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be feedback processes so that the activity of one cell may affect the activity of the others in the vicinity [6]. Donor history, including factors such as infections and stress, is likely to be an important consideration when determining the fraction of time that exposures to RF fields lead to changes in neutrophil activity. The gradient of the electric fields can lead to small drift currents that can increase the rate at which C-AMP molecules strike the surface of the neutrophils [7] and thus can affect neutrophils' behavior. In short, our study indicates that the effects of RF exposure on neutrophil chemotaxis should be considered for further exploration in larger and more controlled studies.

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Complexity Analysis of Arterial Pressure During Periods of Abrupt Hemodynamic Changes

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***Abstract*—In this communication, we estimated the Lempel–Ziv complexity (LZC) on over 40 h of arterial blood pressure (ABP) recordings corresponding to 18 mechanically ventilated animal subjects. In this study, all subjects underwent a period of abrupt hemodynamic changes after an induced injury involving severe blood loss leading to hemorrhagic shock, followed by fluid resuscitation using either lactated ringers or 0.9% normal saline. The LZC metric experienced a statistically significant increase ($p < 0.01$) immediately following the induced injury and a statistically significant reduction following the administration of fluid therapy ($p < 0.01$). These results indicate that LZC of ABP may be useful as a dynamic metric to assess fluid responsiveness.**

***Index Terms*—Arterial blood pressure (ABP), fluid responsiveness, hemodynamic changes, Lempel–Ziv complexity (LZC).**

I. INTRODUCTION

In this study, we analyzed the arterial blood pressure (ABP) signals during periods of abrupt hemodynamic changes using Lempel–Ziv complexity (LZC). This complexity metric was proposed by Lempel and Ziv [1] to evaluate the randomness of finite sequences. It is a nonparametric and simple-to-calculate measure of complexity for 1-D signals that does not require long data segments to be computed [2]. LZC has been widely applied in biomedical signal analysis. It has been used to study the electroencephalogram (EEG) signal of epileptic seizure [3] and the brain information transmission [4]. LZC was also applied to EEG signals in order to quantify the relationship between brain activity patterns and depth of anesthesia [5]. Moreover, EEG and magnetoencephalogram recordings from Alzheimer's disease patients have been analyzed with this measure [6], [7]. LZC has also been used to detect ventricular tachycardia and fibrillation [2], to characterize complexity of DNA sequences [8], and to quantify the complexity in uterine electromyography [9].

In [10], we studied the LZC and its interpretability in terms of classical signal processing concepts such as frequency, number of harmonics, frequency variability of signal harmonics, and signal bandwidth. Our results indicated that LZC was particularly useful as a scalar metric to estimate the bandwidth of random processes.

In this study, we estimated the LZC on over 40 h of ABP recordings corresponding to 18 mechanically ventilated animal subjects. All subjects underwent a period of abrupt hemodynamics changes after an induced injury involving severe blood loss, followed by fluid resuscitation.

Manuscript received July 18, 2006; revised April 27, 2007. This work was supported in part by the Consejería de Educación de la Junta de Castilla y León under Project VA108A06 *Asterisk indicates corresponding author.*

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Digital Object Identifier 10.1109/TBME.2007.901037

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