

Alterations in membrane stability after *in vitro* exposure of human erythrocytes to 2.41 GHz electromagnetic field

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Academic editor: M. Beltcheva | Received 15 November 2022 | Accepted 2 February 2023 | Published 15 May 2023

Citation: Angelova B, Atanasova G, Atanasov N, Paunov M, Gurmanova M, Kouzmanova M (2023) Alterations in membrane stability after *in vitro* exposure of human erythrocytes to 2.41 GHz electromagnetic field. In: Chankova S, Danova K, Beltcheva M, Radeva G, Petrova V, Vassilev K (Eds) Actual problems of Ecology. BioRisk 20: 83–96. <https://doi.org/10.3897/biorisk.20.97616>

Abstract

The growing use of wireless communication devices has been significantly increasing the level of high frequency electromagnetic fields (EMFs) in the environment, which raises a concern for possible deleterious effects on living organisms. Long lasting exposure to low-intensity EMFs can cause effects on the molecular and cellular level, and a number of morphological and physiological changes. The aim of this work was to investigate the effects of 2.41 GHz EMF emitted by wireless communication systems on human erythrocytes after *in vitro* irradiation. The amount of the hemoglobin released from the cells was measured as an indicator for membrane destabilization. Effects of different exposure times (20 min or 4 h) and time elapsed after exposure to 2.41 GHz pulsed or continuous EMFs with different intensities, emitted from a textile (0.213–0.238 V/m) or a dipole (5, 20, 40 and 180 V/m) antenna, were investigated. The obtained results showed that the low intensity EMF had no significant effect on the hemoglobin release from irradiated cells; even a slight tendency for membrane stabilization was noticed 3–4 hours after the end of 20-min exposure to 0.213–0.238 V/m, 2.41 GHz EMF. There was no difference in the effects of continuous and pulsed EMFs. Increased hemoglobin release was observed only during the 4-hour exposure to 180 V/m, 2.41 GHz continuous EMF. Under these conditions, the temperature of the cell suspension had been rising, so we compared the results obtained under EMF with the effects of conventional heating. Moreover, after 1-hour exposure to 180 V/m the released hemoglobin level was a bit higher than the control one but the difference disappears within an hour after terminating the irradiation. In conclusion, the *in vitro* exposure to 2.41 GHz EMF emitted by wireless communication devices with power density below the reference level for population exposure does not change the stability of the cell membrane of human erythrocytes.

Keywords

hemoglobin release, temperature effects, wearable textile antenna, wireless

Introduction

The growing use of wireless communication devices has been significantly increasing the level of high frequency electromagnetic fields (EMFs) in the environment, which raises a concern for possible deleterious effects on living organisms. Long lasting exposure to low-intensity EMFs may cause effects on the molecular and cellular level, and a number of morphological and physiological changes (Camara 2014). Of particular interest is their impact on children and adolescents, who are considered two of the most sensitive and affected groups because they will be exposed to EMFs for the longest time (Bodewein et al. 2022; Schmutz et al. 2022). In communication technology, industry and medicine one of the most commonly used EMF frequency bands is 2.4–2.5 GHz in the microwave range, which is sometimes considered a part of radiofrequency electromagnetic fields (RF EMFs).

Microwave EMF effects can be classified as thermal and non-thermal. Thermal effects are related to energy transfer during interaction between the field and the object, leading to an increase in temperature (Antonio and Deam 2007). The mechanisms of EMF action that are not directly related to temperature changes are not fully understood (Banik et al. 2003; Nguyen et al. 2016 and references therein; Ahortor et al. 2020; Zhao et al. 2021). The effects of EMF on humans can be divided into short-term, such as stress, fatigue, headaches; and long-term, including impaired embryonic development, reduced reproductive capacity, damage to brain tissue, heart problems, cancers, genetic disorders (Kaszuba-Zwoińska et al. 2015; Belyaev et al. 2016). It has been established that radiofrequency irradiation leads to an increase in the formation of free radicals and oxidative stress, which causes disruption of DNA and protein structure, as well as peroxidation of membrane lipids. Changes in gene expression and epigenetic and genetic alterations have been observed (Belpomme et al. 2018). Emerging electromagnetic hypersensitivity has been also receiving increasing attention.

Numerous studies on the effects of EMFs with different frequencies on biological objects with differing degrees of organization have been conducted. The obtained results are contradictory, probably due to differences in the applied irradiation conditions, the objects studied and the detection methods (Banik et al. 2003). The effects of 2.45 GHz microwaves on cell membranes were studied by determining the hemoglobin release and the osmotic resistance of human erythrocytes exposed to different power densities ($0.025\text{--}10.0\text{ mW/cm}^2$) at different irradiation times (Sajin et al. 2000). It was found that at low power densities (0.84 and 1.36 mW/cm^2), the degree of hemolysis increased quasi-linearly with exposure time, while at higher power densities (5 mW/cm^2), this trend reversed after the first 10 hours of irradiation – a protective effect against spontaneous hemolysis caused by blood aging was observed. The

osmotic resistance of exposed erythrocytes (5 mW/cm^2) increased with time, reaching a maximum at the end of irradiation (60 hours), while the osmotic resistance of control cells remained constant. Kouzmanova et al. (2007) found a decrease in the level of released hemoglobin over an hour after 20-minute exposure of erythrocyte suspensions to GSM 900 EMF probably as a result of cell membranes stabilization. Hassan et al. (2010) reported an increased rate of hemolysis after 2.45 GHz EMF irradiation of rats – 1 hour daily for 30 days. In the same study, the level of malondialdehyde, a marker of lipid peroxidation, was significantly increased, and the levels of antioxidant enzymes were significantly decreased. Exposure of male albino rats to EMF with a much lower frequency (50 Hz) caused conformational changes in hemoglobin molecules and significantly reduced serum testosterone, while degenerative changes in the testes were also registered (Salama et al. 2020). 60 Hz EMF exposure of mice was observed to significantly increase micronucleus frequency (Heredia-Rojas et al. 2018). However, a multi-generation study found no harmful effects of RF EMF (1966 MHz) on the fertility and development of mice (Sommer et al. 2009). In rats irradiated with 900 MHz EMF just once for 2 hours or for 4 days, 30 minutes each day, a significant increase in lipid peroxidation of their erythrocyte membranes was observed (Badzhinian et al. 2013). The results were obtained on the first and fifth days after exposure.

Results from *in vitro* experiments with human erythrocytes irradiated with 2.45 GHz EMF showed that short-term (20 minutes) exposure in the reactive near-field of wearable antenna at 6.3 mW input power had a stabilizing effect on the erythrocyte membrane, while long-term exposure (120 minutes) had a destabilizing effect (Atanasov et al. 2022).

Riffo et al. (2021) investigated the effect of EMFs within the frequency band between 1 and 5.9 GHz on yeast growth. A decrease in viability was reported at all applied frequencies. Using transmission electron microscopy, EMF has been found to disrupt the integrity of the cell membrane (membrane permeabilization). When different microorganisms were exposed to 2.45 GHz EMF, an increased permeability of the cell membrane to propidium iodide and dextran particles of various sizes was observed (Ahortor et al. 2020). Entry of propidium iodide was registered in microwave-treated *M. smegmatis* cells but not in cells conventionally heated to the temperature reached by irradiation. Release of DNA from the cells was also reported. 18 GHz EMF was found to induce permeabilization in bacterial and yeast cell membranes as the uptake of high molecular weight dextran (150 kDa) (Shamis et al. 2011) and silica nanoparticles (Nguyen et al. 2015, 2016) was investigated.

Exposure of red blood cells to 18 GHz EMF resulted in cell membrane permeabilization and nanosphere uptake with high efficiency (96% and 46% for 23.5 and 46.3 nm nanospheres, respectively), as demonstrated by scanning electron microscopy, confocal laser scanning microscopy and transmission electron microscopy (Nguyen et al. 2017). Exposure to 2.45 GHz EMF induced a stress response in the hippocampus of rats, evidenced by the presence of heat shock proteins (Yang et al. 2012). Exposure to high-frequency EMFs generated by base stations was associated with an increased risk of developing type 2 diabetes (Meo et al. 2015).

In this study, we investigated effects of EMF used in novel wireless technologies (such as body area or sensors networks, Internet of things, etc. communication systems) on human erythrocyte membranes during and after *in vitro* irradiation. Effects of different exposure periods (20 min or 1, 2, 3 and 4 h) and time elapsed after the exposure to 2.41 GHz pulsed or continuous EMFs differing in intensity emitted by textile (0.213–0.238 V/m) or dipole antenna (5, 20, 40 and 180 V/m) were examined. The amount of hemoglobin released from the cells was measured as an indicator of membrane destabilization.

Methods

Blood material and erythrocyte suspension preparation

The experiments were performed with human erythrocytes, isolated from whole blood drawn from clinically healthy donors (National Center for Transfusion Hematology, Sofia, Bulgaria). Two blood types were investigated: A+ and A-. The EMF treatment was applied between the 5th and 25th day after the drawing while the blood was stored at 10 °C in a refrigerator.

Whole blood samples were centrifuged first at 1500 rpm for 5 min (Eppendorf, Hamburg, Germany), after which the supernatant (blood plasma) and the white blood cells coating was removed and replaced with 0.9% NaCl (saline) solution. Then, the erythrocyte mass was washed twice again with saline, as the cell suspensions were centrifuged for 10 min at 2000 rpm. At the end, the washed erythrocyte mass was collected and its hematocrit was determined by centrifugation in 2–4 capillary tubes for 2 min (Yanetzki TH 11, Germany). The final erythrocyte suspension used in the experiments was obtained by dilution to a hematocrit of 40% with PBS (Sorensen's phosphate buffer – 0.9% NaCl, adjusted to pH 7.4 with Na₂HPO₄/KH₂PO₄). The EMF treatment was carried out in plastic cuvettes filled with 2 ml suspension and covered with Parafilm. Some of the cuvettes were left: as controls isolated from EMF in a metal box; in background irradiation; or in water bath at a temperature of 24, 32 or 38 °C.

Electromagnetic field exposure setups

Two exposure setups were developed to investigate the effects of RF EMF emitted from novel wireless technologies (such as body area or sensor networks, Internet of things, etc.) on human erythrocyte membranes. The first one was designed to test RF EMF exposures from wireless body area network devices. The RF EMF was generated with an XBee S1 RF module (Digi International Inc., Thief River Falls, MN, USA) connected to a microwave solid-state amplifier (CBA 9429, AMETEK CTS Europe GmbH, Kamen, Germany). The RF module was controlled by a personal computer to emit a Zigbee-like signal (1 ms between the packets) at 2.41 GHz. The signal was transmitted using a wearable textile polyester substrate antenna (Atanasova and Atanasov 2020),

connected via a 6 dB attenuator to the microwave solid-state amplifier, as shown in Fig. 1. The erythrocyte suspensions were placed in the far-field region of the antenna. The erythrocyte suspensions were exposed to EMF with power density 120–150 $\mu\text{W}/\text{m}^2$ and intensity 0.213–0.238 V/m, measured with a battery-operated E-field probe (HI-6006, ETS-Lindgren, Cedar Park, TX, USA). Since the two EMF parameters are proportional in the far-field region, only the intensity will be presented further. The effect of the pulsed EMF on erythrocyte membranes was compared to that of continuous EMF exposure. For that purpose, the erythrocytes were exposed to a 0.213–0.238 V/m continuous wave EMF at 2.41 GHz as well (see Fig. 1).

The second experimental setup was designed to test RF EMF with higher electric field intensity. The RF EMF was generated with a microwave generator (SMB100A, Rohde & Schwarz GmbH & Co. KG, Munich, Germany) connected to a microwave solid-state amplifier (FLG-50F, Frankonia, Heideck, Germany). The microwave generator was tuned to generate a pulse-modulated signal (pulse period 4.608 ms, pulse width 2.304 ms, 217 Hz) at 2.41 GHz. The signal was transmitted using a half-wave dipole metal antenna connected via a coaxial cable to the microwave solid-state amplifier. The erythrocyte suspensions were placed in the far-field region of the antenna on a Styrofoam in four positions differing in intensities: FF1 (180 V/m), FF2 (40 V/m), FF3 (20 V/m), and FF4 (5 V/m), as shown in Fig. 2. The electric field at each position was measured as in the first setup. Exposures were performed in a semi-anechoic chamber for both setups. The temperature of the samples during irradiation was monitored with an infrared thermal camera FLIR E5 (Teledyne FLIR, Wilsonville, OR, USA).

During the experiment the background control samples were placed in rooms adjacent to the semi-anechoic camera. The ambient EMF in those rooms was measured. Power density varied in the range of 36–72 $\mu\text{W}/\text{m}^2$ (0.116–0.164 V/m). The ambient EMF values were lower than those applied to erythrocyte suspensions in the semi-anechoic chamber.

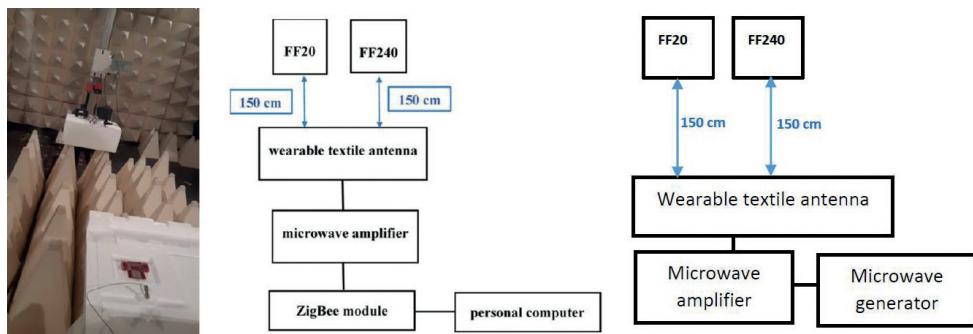


Figure 1. Experimental design 1. Two plastic cuvettes filled with 2 ml erythrocyte suspensions (hematocrit 40%) were located at 150 cm distance from the textile antenna (in the far field region) and irradiated for 20 (FF20) or 240 minutes (FF240) with pulsed or continuous EMF. Input power to the antenna was 450 mW, electric field intensity – 0.213–0.238 V/m. Left: photograph of the general setup; Center: scheme representing pulsed EMF setup; Right: scheme representing continuous EMF setup.

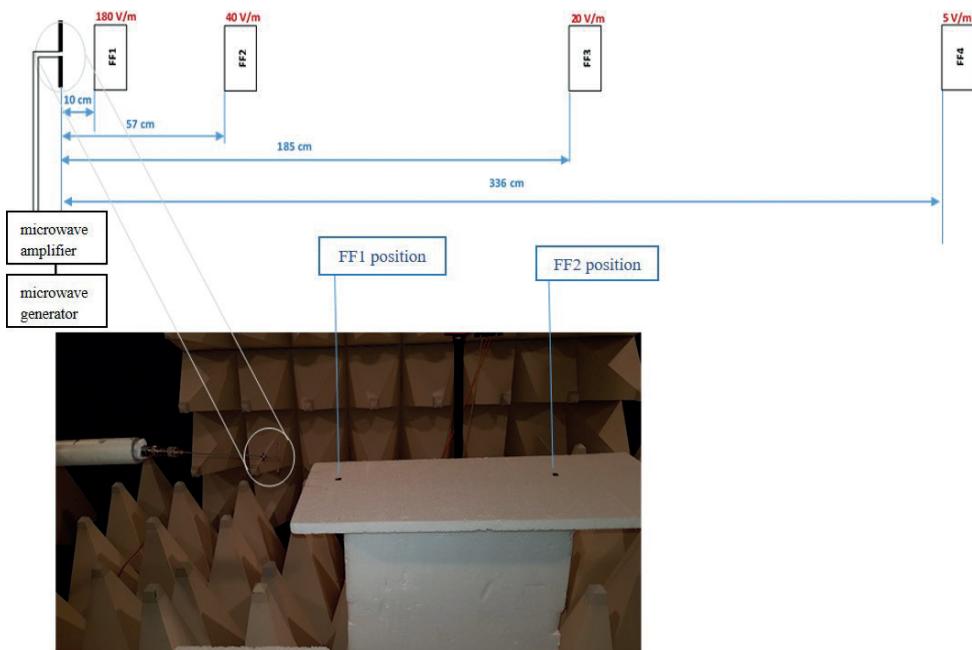


Figure 2. Experimental design 2. Four plastic cuvettes filled with 2 ml erythrocyte suspensions (hematocrit 40%) were located at different distances from the dipole antenna (in the far field region) at four intensities: FF1 (180 V/m), FF2 (40 V/m), FF3 (20 V/m), and FF4 (5 V/m), as shown on the scheme and the photograph. Input power to the antenna was 50 W.

Hemoglobin release measurement

The release of hemoglobin was estimated spectrophotometrically by measuring the absorbance at 413 nm (maximum for hemoglobin) of a supernatant solution (Spekol 11, Carl Zeiss Jena, Germany). The supernatant solution was prepared as 100 µl of the investigated erythrocyte suspension was added to 1.3 ml of PBS followed by centrifugation for 15 s at 12000 rpm. The concentration of the released hemoglobin in the 40% hematocrit experimental sample was calculated using the formula:

$$c = \frac{A \times V_2}{\varepsilon \times l \times V_1}$$

where c is hemoglobin concentration, µmol/l; A – absorbance; ε – molar extinction coefficient for hemoglobin at 413 nm ($0.12 \text{ l.} \mu\text{mol}^{-1} \cdot \text{cm}^{-1}$); l – optical path length through the spectrophotometrically measured sample (1 cm); V_1 – volume of added erythrocyte suspension (100 µl) and V_2 – final measured sample volume (1400 µl).

Statistics

The results presented in this study are average values \pm standard errors calculated from 3–7 independent repetitions of each experimental variant.

Results

Exposure to the textile antenna

The hemoglobin release from erythrocytes in suspensions with hematocrit 40% was investigated for 5 hours (at 1-hour interval) after 20-min exposure to pulsed 2.41 GHz EMF with intensity 0.213–0.238 V/m (Fig. 3A). Simultaneously, the hemoglobin release in control untreated suspensions was measured. The control samples were placed in the semi-anechoic chamber during the exposure but were shielded from EMFs in a metal box. Thus both the control and the EMF-treated cells were at the same temperature during irradiation. No heating was registered for the EMF exposed samples. After the end of the treatment, both sample types were placed in a water bath at 24 °C. Both EMF-treated and control cells displayed a tendency for released hemoglobin increase with time passing after exposure but statistically significant changes were not registered even after 5 hours. No statistically significant differences in the quantity of hemoglobin between exposed and unexposed suspensions were observed.

Further, erythrocytes were treated with continuous EMF without changing the other irradiation parameters. The obtained results are presented in Fig. 3B. Again, no statistically significant difference in the released hemoglobin between control and treated samples was found due to the high variance (standard errors) of the values.

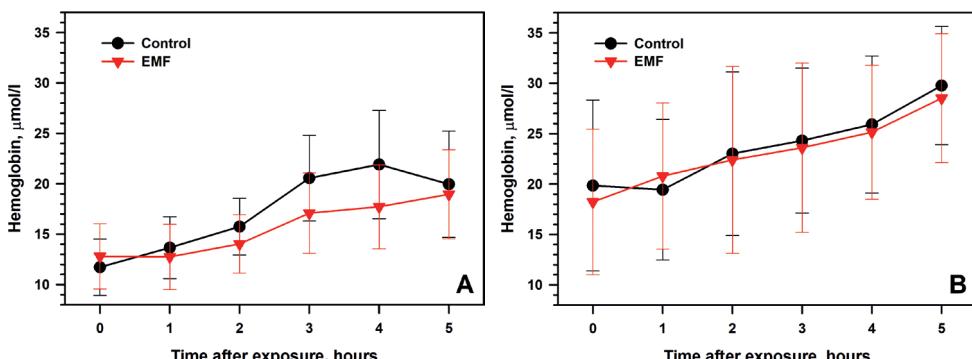


Figure 3. Hemoglobin release after 20-min irradiation of human erythrocyte suspensions with 2.41 GHz EMF. Source: textile antenna, intensity 0.213–0.238 V/m **A** pulsed EMF (1 ms between the pulses) applied **B** continuous wave EMF applied. Control: erythrocyte suspensions shielded from EMFs.

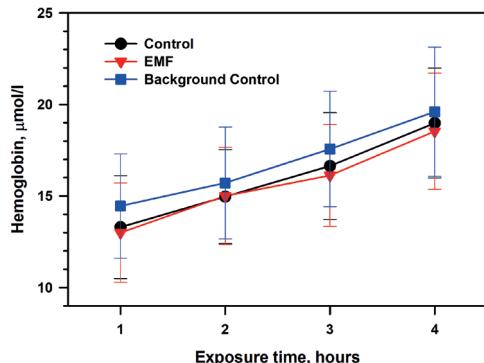


Figure 4. Hemoglobin release during 4-hour exposure of human erythrocyte suspensions with 2.41 GHz pulsed EMF. Source: textile antenna, intensity 0.213–0.238 V/m. Control: samples isolated from EMFs; Background Control: samples at 0.116–0.164 V/m background EMF.

Since no EMF effects were registered after 20-min irradiation from textile antenna and because the communication devices operate with EMF pulses, we continued our experiments with longer (4-hour) pulsed EMF exposures during which the hemoglobin release was measured every hour. Again, a control sample in a metal box was used. Moreover, two background controls were placed in two rooms during the experiment at 24–26 °C ambient temperature. The results from these two samples were averaged and presented as background control. No statistically significant differences between the control, background control, and EMF-treated samples were observed even after 4 hours (Fig. 4). Heating was not registered for the irradiated sample, so its temperature was the same as the control samples. However, in all the conducted (7) individual experiments a tendency for higher values in background control was observed which is also noticeable from the averaged data. During the experiments, intensity of 0.116–0.164 V/m was measured for the background EMF. For comparison, just after the end of the 20-min exposure at the same conditions the released hemoglobin was 12.8 μmol/l, and 4 hours after terminating the irradiation – 17.73 μmol/l, while after 4-hour continuous EMF treatment it was 18.54 μmol/l. Thus, at the applied EMF parameters the longer exposure did not affect strongly the integrity of the erythrocyte membranes *in vitro*.

Exposure to the dipole antenna

All the experiments conducted with the textile antenna show no effect of EMF on the stability of erythrocyte membranes. In search for effect, an antenna, allowing higher intensity emission, was used. The effect of 2.41 GHz EMF emitted by a half-wave dipole antenna with an output power of 50 W (pulse period: 4.608 ms, pulse width: 2.304 ms) on erythrocyte suspensions was investigated for 4 hours. Samples were placed in far-field at 4 positions from the antenna with different electric field intensities: 5, 20, 40 and 180 V/m. From Fig. 5A it is evident that the erythrocytes at the 5,

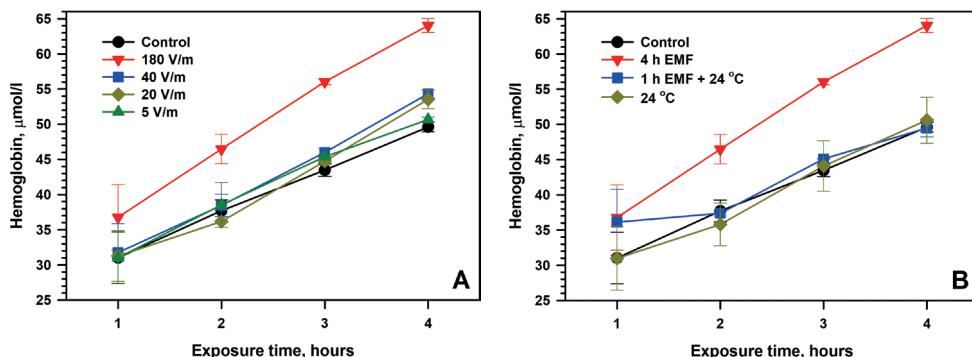


Figure 5. Hemoglobin release during 4-hour irradiation of human erythrocyte suspensions with 2.41 GHz pulsed EMF. Source: dipole antenna, pulse period: 4.608 ms, pulse width: 2.304 ms. **A** different intensities applied: 5, 20, 40 and 180 V/m **B** sample which 180 V/m EMF exposure was interrupted after 1 h, compared to 4-hours long uninterrupted 180 V/m treatment. Control: erythrocytes shielded from EMF; 24 °C: cells incubated in water bath at 24 °C for 4 hours.

20 and 40 V/m intensities released hemoglobin similarly to the control for the first 3 hours. Values of 20 and 40 V/m samples became higher than 5 V/m and control levels just after 4 hours. The highest intensity (180 V/m) caused the greatest hemoglobin release, as a significant difference compared to the control appeared at the second hour and continued throughout the exposure time.

A significant temperature increase to 32 °C was registered in the samples exposed to 180 V/m. Since it is known that the biological effects of EMFs are at least partially due to heating, the hemoglobin release after conventional heating was investigated. The concentration of the released hemoglobin after 4-hour incubation at 24, 32 and 38 °C in a water bath was 218±160, 311±235, 277±209 µmol/l, respectively. For the large standard errors, we could not determine a significant temperature-dependent change, just a tendency for heat-induced increase.

In addition, the stability of erythrocyte membranes after exposure to high-intensity EMF was examined and compared with membrane stability changes during the EMF action. Two cuvettes with erythrocyte suspensions were placed under irradiation with 180 V/m intensity EMF. After one hour, one of the samples was moved from the irradiation spot into a water bath at 24 °C while the other was left under treatment. A control sample in a metal box and a 24 °C incubated control were used. Released hemoglobin was measured simultaneously for all samples for 4 hours (Fig. 5B). Under the uninterrupted (4 h) EMF exposure, the quantity of released hemoglobin increased linearly with time. The hemoglobin amount in the interrupted treatment sample did not change during the first hour after removing the EMF irradiation, approaching control levels at the 2nd hour, but subsequently increased equally with control values over time. There were no differences in the released hemoglobin concentrations between the control sample in a metal box and 24 °C sample.

Discussion

The rapid development of wearable wireless sensor networks, and the fact that emitted EMFs may have impact not only on the people wearing such sensors, but also on the people around them, leads to an increased interest in the biological effects. In order to clarify possible effects of EMF exposure in the far-field region on cell membrane we conducted experiments with a small wearable textile antenna and with a dipole antenna.

The selectively permeable cell membrane allows the transport of some soluble substances across it and prevents the passage of others. Thus, the membrane is involved in the control of cell volume and integrity. When it comes to red blood cells, this is of great clinical importance. The process in which the integrity of the erythrocyte membrane is impaired and the intracellular protein hemoglobin is released into the environment is called hemolysis. It can result from normal cell aging or be induced by various biotic and abiotic factors (Goodhead and MacMillan 2017). The accumulation of free hemoglobin in the body can cause heart disease or kidney stones (Prastalo et al. 2003). Various literature data show that exposure of erythrocytes to EMF – both *in vitro* and *in vivo*, leads to a change in the stability of their cell membranes (Kiel and Erwin 1984; Kouzmanova et al. 2007).

Our results showed there was practically no change in the quantity of released hemoglobin for 5 hours after 20-min exposure of human erythrocytes to 0.213–0.238 V/m 2.41 GHz pulsed or continuous EMF emitted by the textile antenna. Slight variations between pulse-treated and control suspensions were observed at 3rd and 4th hour, hinting at possible tendency for membrane stabilization, similar to the results obtained by Kouzmanova et al. (2007). In both samples, the average hemoglobin values rose with the incubation time as to be expected because of cell degradation during storing erythrocytes at room temperature in absence of nutrients in the medium. However, the standard errors of the presented released hemoglobin concentrations are very large as a result of variation between the values measured in each replication of the experiment. That variance should be attributed mainly to the *in vitro* aging of the blood, respectively erythrocytes, i.e. the time elapsed between drawing the blood and conducting the experiment, as Kouzmanova et al. (2006) observed increasing level of hemolysis in erythrocytes from “aged” blood. Individual characteristics of blood donors should be also expected to introduce high variance.

A tendency for higher hemoglobin values in background control compared to the shielded control and 4-hour EMF exposed samples was noticed, which cannot be explained by the influence of 0.116–0.164 V/m background EMF. This intensity is lower than the experimentally applied 0.213–0.238 V/m. The intensity of the background EMF radiation varies throughout the day, depending mainly on the level of communication systems usage by the population. On the other hand, the background EMF may vary in frequency as well, and mild discrepancies between the temperature in the semi-anechoic chamber, where control and treated samples were placed, and the laboratories, where the background controls were placed, were pos-

sible to occur (in the range of 2 °C). The simultaneous action of all those factors may explain the observed results.

On the basis of the maximal levels of irradiation defined in IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz (2006), four positions were chosen in the dipole antenna exposure setup, ensuring EMF intensity values, significantly higher than those applied during the textile antenna experiment. The allowed maximal power density in controlled environment (an area in which workers are subject to control and accountability – in radio transmitters, installers of base stations, etc.) is 80.3 W/m², which corresponds to the intensity of 174 V/m. For the general population (people of all ages with different health statuses) a power density of 10 W/m² (61.4 V/m) is accepted as permissible. At the first sample position, the intensity was slightly higher than the maximally allowed for a controlled environment (180 V/m), while the other three positions had values (40, 20 and 5 V/m), resembling realistic cases of general population exposure.

There seemed to be slight alterations in the cell membrane permeability leading to a tiny increase of the released hemoglobin after 1-hour exposure to 180 V/m, but one hour after the end of irradiation, the membrane fully recovered. The properties of the biological membranes depend directly on the state of the membrane proteins. Upon their functioning, proteins undergo different conformational changes. They have many charged chemical groups, taking part in catalytic, regulatory, transport and aggregation processes, which can be influenced by EMF (Bucci et al. 2006). Such changes could result in the observed effects on hemoglobin release.

It is supposed that the thermally induced hemolysis includes 3 types of processes: 1) inactivation of vital enzymes and denaturation of structure proteins, 2) formation of lytic agents in the blood plasma and 3) melting of membrane lipids (Gershfeld and Murayama 1988). The structure of spectrin – a cytoskeletal protein, is not altered at temperatures under 45 °C, so the cytoskeleton impairment cannot be related to cell lysis under the examined experimental conditions. The highest temperature investigated (38 °C) is optimal for the functioning of the cellular enzymes. Hence their inactivation is not a possible explanation for the hemolysis. The temperature of the phase transition of the erythrocyte membrane from gel to liquid crystal is far below 37 °C so lipid melting is not possible to contribute to the observed hemoglobin release (Gershfeld and Murayama 1988). The activation energy of autohemolysis of red blood cells in the range 4–37 °C is significantly less than that at a temperature higher than 37 °C. This means that the change in the limiting stage of hemolysis occurs at 37 °C. Oxidative processes were found to be essential in autohemolysis in the range of 20–37 °C (Chernitskiĭ and Iamaĭkina 1996). Between 38 and 45 °C a process different from protein inactivation is responsible for hemolysis – mechanism based on the concept of the critical bilayer assembly temperature of cell membranes.

Our results could not differentiate thermal from non-thermal effects of EMF on hemolysis at 180 V/m *in vitro*. We plan future experiments to elucidate such differences, i.e. whether non-thermal effects exist at permissible EMF exposures and what are their mechanisms.

Conclusion

In vitro irradiation with 2.41 GHz EMF emitted from wireless communication devices with power density / electric field intensity below the reference level for the general population according to IEEE (2006) does not change the stability of the human erythrocyte cell membrane for up to 4 hours of exposure.

Acknowledgements

This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, grant number KP-06-H57/11 from 16th November 2021 “Antenna structures for new energy sources in next-generation wireless networks”, leader Assoc. Prof. Gabriela Atanasova, South-West University “Neofit Rilski”. These results were reported at the International Seminar of Ecology – 2022, Actual Problems of Ecology. September 29–30 2022, Sofia.

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