Biological effect of ELF electric field in blood aggregation

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Abstract

Among the various biological effects of extremely low frequency (ELF) electric fields, increased peripheral blood flow occurs when a human body is exposed to an electric field. To elucidate this phenomenon, we investigated changes of red blood cell (RBC) aggregation. Fifteen volunteer participants stopped taking food at least 10 hr including sleeping period before the experiment. Blood was sampled according to a predetermined schedule, and put on a slide glass for microscopic observation. Then microscopic images of RBCs were analyzed using image-processing software. The RBCs were classified into several groups: normal, aggregated, megacytic, microcytic, and degenerated RBCs. For this study, we observed the ratios of normal and aggregated RBCs as a parameter related to the blood flow change. Each participant was exposed to the electric field using a therapeutic electric field exposure instrument (Healthtron HES-30; Hakuju Institute for Health Science Co., Ltd.) that applied high voltage (30 kV) beneath the participant’s soles of the feet. The participant’s body was electrically floated from the ground with the insulating chair. A high electric field (20–250 kV/m) was generated between the participant body and the grounded environment.

Each participant kept sitting calmly on a comfortable chair of the field exposure instrument for 5 min before the experiment started. Then blood was sampled, and the 30-min-long exposure/sham-exposure period started. At the end of the period, blood was sampled. The participant remained seated calmly on the chair for one hour. During this post-exposure period, blood was sampled every 15 min. Blood aggregation is expected to decrease with sufficient water intake by the participant. Therefore, the combined experiment of sham exposure was conducted with water intake. With our measurement system, the RBC aggregation was stable in the sham-exposure period, but it decreased with water intake. Results of this experiment confirmed the stability and sensitivity of our measurements. Then, the participant was exposed to the field and the blood samples were analyzed. Comparison between the results of sham-exposure and exposure cases revealed that blood aggregation increased about 20% because of field exposure.

To elucidate the mechanism underlying this phenomenon, we conducted another experiment of in vitro exposure of the electric field. The electric field was applied to the blood sample itself on the slide glass under a microscope. The field strength was controlled so that the induced current density in vitro was the same level as that in vivo. Consequently, the ratio of the aggregated RBCs tended to increase with in vitro exposure. Results suggest that the aggregation increase in the field exposure is attributable not only to the physiological change in the participant body but also to the physical change of RBCs or their environment. The validity of this results and the relation between blood aggregation and blood flow must be investigated further.

Keywords: ELF electric field, Blood flow, Red blood cells (RBC’s), Aggregation, Human exposure, Biological effects.
Biography

Miki Kanemaki was born in Bibai, Hokkaido, Japan, in 1992. She received a B.S. degree in Clinical Engineering from Hokkaido University of Science, Sapporo, Japan, in 2015. She is a graduate student of the Division of Biomedical Engineering in Hokkaido University of Science, Sapporo, Japan. She has been active in research on biological effects of ELF (0–300 Hz) electric fields.

Hisae Odagiri - Shimizu received her Ph.D. degree from Hokkaido University, Sapporo, Japan, in 2002. She is currently a professor of Department of Clinical Engineering, Hokkaido University of Science, Sapporo, Japan. She has been engaged in studies of biomedical engineering, particularly the biological effects of electromagnetic fields. She is a member of IEEE, Japanese Society for Medical and Biological Engineering, and the Institute of Electronics Information and Communication Engineers.

Masataka Kitama received the B.S. degree from Hokkaido Institute of Technology, Sapporo, Japan in 1990, 1992 and 1995 he received the M.S. and the Ph.D. degrees in Biomedical Engineering, respectively, from Hokkaido University, Sapporo, Japan. From 1995 to 1996 he was a Research Fellow of the Japan Society for the Promotion of Science. Since 1996 he has been working for Hokkaido Science University, former Hokkaido Institute of Technology, as a Lecturer, an Assistant Professor, an Associate Professor and a Professor. He was an academic visitor at the Department of Medical Physics and Bioengineering, University College London in 2005. He was awarded by Japanese Society for Medical and Biological Engineering several times, including Sakamoto Award for the outstanding paper in 1994. He has been engaged in the studies of biomedical engineering including those of wave propagation in biological media, optical imaging for biomedical applications and development of management technique of arteriovenous fistula in hemodialysis. He is a member of the Institute of Electronics, Information and Communication Engineers, Japan. He has been a board member of Japanese Society for Medical and Biological Engineering.
Biography

Masaji Yamashita is a Professor of Clinical Engineering at Faculty of Health Sciences, Hokkaido University of Science, Sapporo Japan. He is engaged in the researches on the bioelectromagnetics, the biotelemetry and the affective engineering, etc. Currently, his research interests are the affective engineering and the autonomic nervous system. He is a member of Japan Society of Kansei Engineering, Japanese Society for Medical and Biological Engineering, Japan Society of Physiological Anthropology, Institute of Electronics, Information and Communication Engineers and Society of Instrument and Control Engineers.

Hiroko Miura is an associate professor of English at Hokkaido Institute of Technology (Hokkaido University of Science). Her research interests include TESOL, especially in cross-cultural communication and affects of mother tongue on foreign language learning. She has publications on the area of TESOL and ESP.

Koichi Shimizu received the B.S. degree from Hokkaido University, Sapporo, Japan, in 1973. In 1976 and 1979 he received the M.S. and the Ph.D. degrees, respectively, from University of Washington, Seattle, USA. From 1974 to 1979 he was a Research Associate in the Center for Bioengineering and the Department of Electrical Engineering, University of Washington, Seattle. From 1979 to 2016, he was an Assistant Professor, an Associate Professor and a Professor in Hokkaido University, Sapporo, Japan. He is currently a Professor of Graduate School of Information, Production and Systems, Waseda University, Kitakyushu, Japan. He has been engaged in the studies of biomedical engineering including those of wave propagation in biological media, optical imaging for biomedical applications, biotelemetry and biological effects of electromagnetic field. He is a member of IEEE, OSA, ISOB and engineering societies in Japan. He served as an Associate Editor of IEEE Transactions on Information Technology in Biomedicine 1999 – 2007. He has been a board member of ISOB since 1991.
1. Background
2. Experiment of *in vivo* exposure
3. Experiment of *in vitro* exposure
4. Conclusion
1. Background

extremely low frequency (ELF: 0-300 Hz) electric field
1. Background

However, the mechanism has not been elucidated completely.

- Investigated the human physiological responses in ELF electric field exposure.
1. Background

RH 50% 250 kV/m  

RH 90% 200 kV/m  

AC field

Blood Flow (a. u.)

Exposure

1 min
Blood flow change

We could not detect apparent change in the expansion/contraction of the peripheral blood vessels.

The purpose of this study:
Investigate the aggregation change of RBCs as another mechanism for the blood flow change
1. Background
2. Experiment of *in vivo* exposure
3. Experiment of *in vitro* exposure
4. Conclusion
2. Experiment of *in vivo* exposure

- **Subjects**
  - ✔ sex: male volunteers
  - ✔ age: 21-24
    - Stopped taking food at least 10 hours
    - Wore only short pants

- **Laboratory setting**
  - ✔ temperature: 25-27°C
  - ✔ humidity: 70-80%

This study has been approved by the ethics committee.
2. Experiment of \textit{in vivo} exposure

- **Equipment**

  Therapeutic instrument commercially available
  High voltage (30kV) beneath the subject’s soles of the feet
2. Experiment of *in vivo* exposure

**Blood sample**

Subjects puncture ring-finger by themselves.

Classification: normal, aggregated, megacytic, microcytic, and degenerated Red Blood Cells
2. Experiment of *in vivo* exposure

- **Time-course of experiment**

  **sham exposure of electric field**
  - 5 min
  - 30 min
  - 15 min each
  - rest 1
  - sham exposure
  - rest 2
  - rest 3
  - rest 4
  - rest 5

  **sham exposure with water intake**
  - 5 min
  - 20 min
  - 30 min
  - 15 min each
  - rest 1
  - water intake
  - sham exposure
  - rest 2
  - rest 3
  - rest 4
  - rest 5

  **electric field exposure**
  - 5 min
  - 30 min
  - 15 min each
  - rest 1
  - exposure
  - rest 2
  - rest 3
  - rest 4
  - rest 5

  ↑ blood sampling and observation
2. Experiment of *in vivo* exposure

Temporal change of aggregation in sham exposure

![Graph showing the temporal change of aggregation in sham exposure. The x-axis represents time (min) with values at 0, 15, 30, 45, and 60, and the y-axis represents the ratio of RBC's (%). The graph compares normal and aggregation states before and after sham exposure.](image)

- **Normal** and **Aggregation** states are indicated.
- **Before sham exposure** (0 min) shows a normal ratio.
- **After sham exposure** (15 to 60 min) shows an aggregation ratio.
- The data points are represented with error bars, indicating variability.
- The graph includes a note on N.S. (not significant) with N=15.
2. Experiment of *in vivo* exposure

**Change of aggregation with water intake**

- **Ratio of RBC's (%):**
  - Normal: 100%
  - Aggregation:
    - 1%
    - 10%

<table>
<thead>
<tr>
<th>Water Intake</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>after</td>
<td></td>
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</tr>
</tbody>
</table>

**Significance:**
- **P<0.05**
- **P<0.01**

**N=14**
2. Experiment of *in vivo* exposure

aggregation change in field exposure

![Graph showing aggregation change in field exposure](image)

**Ratio of RBC's (%)**

- Before exposure: Normal (blue circles) and Aggregation (red circles)
- After exposure (0, 15, 30, 45, 60 min): Normal (blue circles) and Aggregation (red circles)

**Notes:**
- ****: P < 0.01
- N = 15
2. Experiment of *in vivo* exposure

Possible Mechanisms for aggregation change
1. Physiological change inside the body
2. Physical change of blood condition

Observable in experiment of *in vitro* exposure?
1. Background
2. Experiment of *in vivo* exposure
3. Experiment of *in vitro* exposure
4. Conclusion
3. Experiment of *in vitro* exposure

- Equipment

**Insulated metal electrodes**

Current density in human body was 6-75 mA/m$^2$
3. Experiment of *in vitro* exposure

**Sham Exposure**
- 5 min each
- **Rest 1**
- **Rest 2**
- **Sham Exposure**
- **Rest 3**
- **Rest 4**

**Before Sham Exposure**

**After Sham Exposure**

**Exposure**
- 5 min each
- **Rest 1**
- **Rest 2**
- **Exposure**
- **Rest 3**
- **Rest 4**

**Before Exposure**

**After Exposure**

**Blood Sampling**

**Observation**
3. Experiment of *in vitro* exposure

*In vitro* sham exposure of electric field

![Graph showing the ratio of RBC's (%)]

- **Normal**
- **Aggregation**

**N.S.**

N=15

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Before sham exposure</th>
<th>After sham exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Normal: 95%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Normal: 97%</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Normal: 98%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Aggregation: 6%</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Aggregation: 8%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Aggregation: 4%</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Aggregation: 2%</td>
<td></td>
</tr>
</tbody>
</table>

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3. Experiment of *in vitro* exposure

*In vitro* exposure of electric field

![Graph showing the ratio of RBCs (%) before and after exposure to electric field. The graph compares normal and aggregation states, with exposure time indicated in minutes.](image-url)

- Normal
- Aggregation

Ratio of RBC's(%)

- Before exposure
- After exposure

N.S.

N=15

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3. Experiment of *in vitro* exposure

*In vitro sham exposure*

*In vitro* exposure

The average values show the same tendency as in vivo case.

Normal RBCs decreased and aggregated RBCs increased.
Mechanism of blood flow change in field exposure

RBC aggregation was analyzed.

1. RBC aggregation **increased** in ELF field exposure.
2. Clear increase in *in vivo* exposure.

**New mechanism for biological effect of ELF electric field**
References