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Why modulated electrohyperthermia (mEHT) destroys the rouleaux formation of erythrocytes?

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ABSTRACT

Our aim in this paper is to describe systemic observations of blood samples before and after modulated electrohyperthermia (mEHT) treatment, to clarify its systemic effect on blood. The method is also feasible to control the efficacy of the mEHT treatment process.

Keywords

oncothermia, rouleaux formation, erythrocytes, electric field, electrophoretic force, disaggregation, mice, human

INTRODUCTION

Observations and explanations of the rouleaux phenomenon have induced much debate since its discovery. Erythrocytes often form rouleaux-patterns (RP) caused by surface adhesion between them. This is a way to decrease the surface energy (Dirichlet-principle, [1]) connected to their surface area. The aggregation of erythrocytes is a prominent feature in humans as well as other species. The rheological parameters of blood are mainly the apparent viscosity at low shear rates and the red blood cell (RBC) sedimentation rate. The aggregation also correlates with oxygen consumption [2].

In vitro studies have shown that aggregation of blood increases as shear rate decreases. Aggregation also depends on hematocrit, the concentration of macromolecules in the plasma or suspending medium [3] and on the presence of high molecular weight polymers, such as plasma proteins or dextrans, which aggregate to form RP and rouleaux networks [4]. However, the circumstances in which aggregations occur are not well understood. Correlations of aggregation parameters with C-reactive proteins and fibrinogen were found in unstable angina, acute myocardial infarctions and bacterial infections [5].

RP has an important role in the hydrodynamics of blood. The viscosity of the suspension grows with the average size of the dispersed solids at a constant volume and concentration of dispersion. As a consequence RP could have a control role in blood-flow, as their presence limits blood-flow in the large vessels due to their viscosity, while smaller sizes could increase the flow in the capillaries.

The reason why a RP is formed in large vessels is that blood is not a simple (Newtonian) liquid but a Bingham-type [6], with a threshold value (shear stress, share rate) that starts the flow. In mathematical form it is

$$\tau = \tau_f + \eta D \qquad (1)$$

where τ is the stress in the liquid, τf is the flow-stress and η and D are the viscosity and the shear rate respectively. The stress of the flow in the axis of the tube is zero and increases linearly to its maximum at the inner surface of the vessel. The tendency of bias towards aggregation increases with decreasing blood-flow in the venous network; the blood-flow tends to Bingham-liquids [7] by increasing deviation from the classical Newtonian liquids [8], (see figure 1). Blood rheology has a crucial role in RP formation, [9].

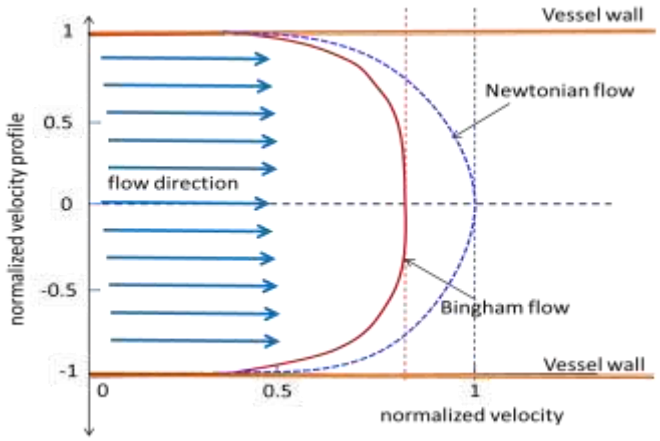

Figure 1. The blood-flow velocity profiles. Newton's flow and Bingham's flow (cork profile), depending on the various concentrations of erythrocyte aggregates and surfactants of filaments [10].

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Volume 9 Number3
Journal of Advances in Biology

Geometrical criteria decide the RP and network of the aggregation [11]. The aggregates may branch, forming trees, and when RP grow large they may contain rings or loops and form network-like structures [12]. The shape of the erythrocytes in the RP is the function of their diameter [13] together with adhesion and area differences (see Figure 2) [14].

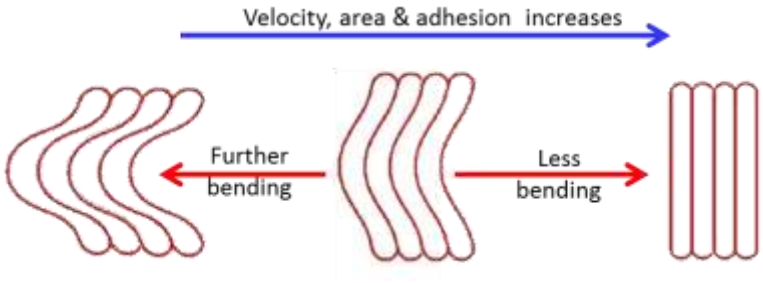


Figure 2. Bending of erythrocytes in the RP formations depending on various environmental and internal parameters.

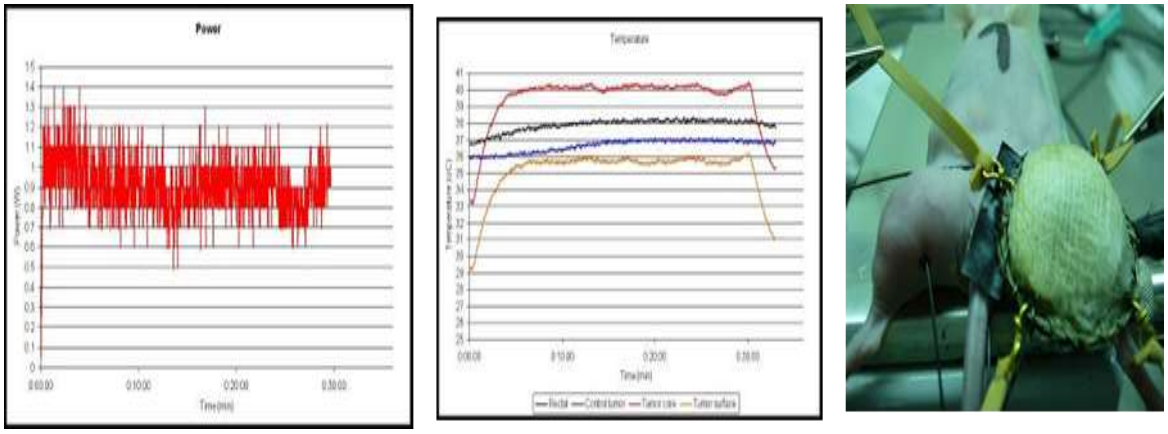
The low shear rates in tumor vessels may facilitate RP formation [10]. However, despite the large shear rates present in microcapillaries, fibrinogen or synthetic polymer dextran content leads to enhanced RP formation, even at low concentrations of haematocrits [15].

Thrombocytes influence the aggregation of erythrocytes, which is enhanced by higher platelet concentrations [16]. The activation of platelets leads to clot formation, which is a double-edged sword; it is lifesaving in the wound healing process but on the other hand clot formation causes the majority of deaths in thrombus induced strokes [17]. RP formation correlates with inflammation states as well [18] and in this way it may also correlate with cancerous states, which we show below. The study of the electrical properties of red blood cells and their RP formation can contribute to immunohematology practice [19].

METHODS

The treatment was performed using modulated electrohyperthermia (mEHT, trade name oncothermia) with a special laboratory device for small animals (EHY110, Oncotherm GmbH, Germany). The method was an impedance based capacitive coupled, amplitude modulated radiofrequency treatment [20]. The carrier frequency was 13.56 MHz, the modulation was a time-fractal pattern [21]. mEHT selectively targets the rafts on the membranes of malignant cells [22]. The nano-selection is based on certain deviations in the metabolic-processes of cellular connections and in the organising patterns of malignant cells compared to their healthy counterparts. The cell-killing mechanism is connected to the intensive, but very local, nano-range energy absorption, which is selectively delivered by electrodynamic conditions.

Blood samples of nude mice were studied before and after the oncothermia treatment. The mice (Balb/C nu/nu) were xenografted with the human HT-29 colorectal carcinoma cell-line in both femoral regions symmetrically in the heterotopic subcutaneous. The electrode was flexible and the applied power spectrum and temperature plot are shown in Figure 3.



a. b. c.

Figure 3. Typical experimental details from left to right: power control, temperature pattern and electrode arrangement

The mice (ten animals) are shown for reference in Figure 4. The treatment consisted of a single shot for 30 minutes, reaching and keeping 40°C constantly in the tumor, while the other tumor (always the left one) was not treated but instead used as a reference (modelling a non-treated distant metastasis in the animal.) Blood samples from the mice were carefully collected from the tails (venipuncture in the tail vein) of the mice under anesthesia.

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Figure 4. The set of mice involved in this study

Blood samples from human volunteers were also collected. The human donors had suffered various malignant diseases. The human samples were peripheral, obtained from capillaries in the finger-pad. Samples of venous blood from humans were also collected for comparison. The blood collection was done immediately before and immediately after mEHT treatment, as well as being systemically performed during subsequent treatments in humans. The samples were freshly measured by dark-field microscopy (the slide-holder table was not heated). The pictures were archived using high resolution photo or video techniques.

RESULTS

Before the treatment, RP formations in blood samples were observed in 40% of the investigated animals (see Figure 5.) and in the majority of the human individuals. In all cases where the RP formation was found, the mEHT treatment changed the RP network and a large proportion of the samples were free of erythrocyte aggregates. This phenomenon was independent of the treatment localisation and also from the venous or arterial origin of the blood sample.

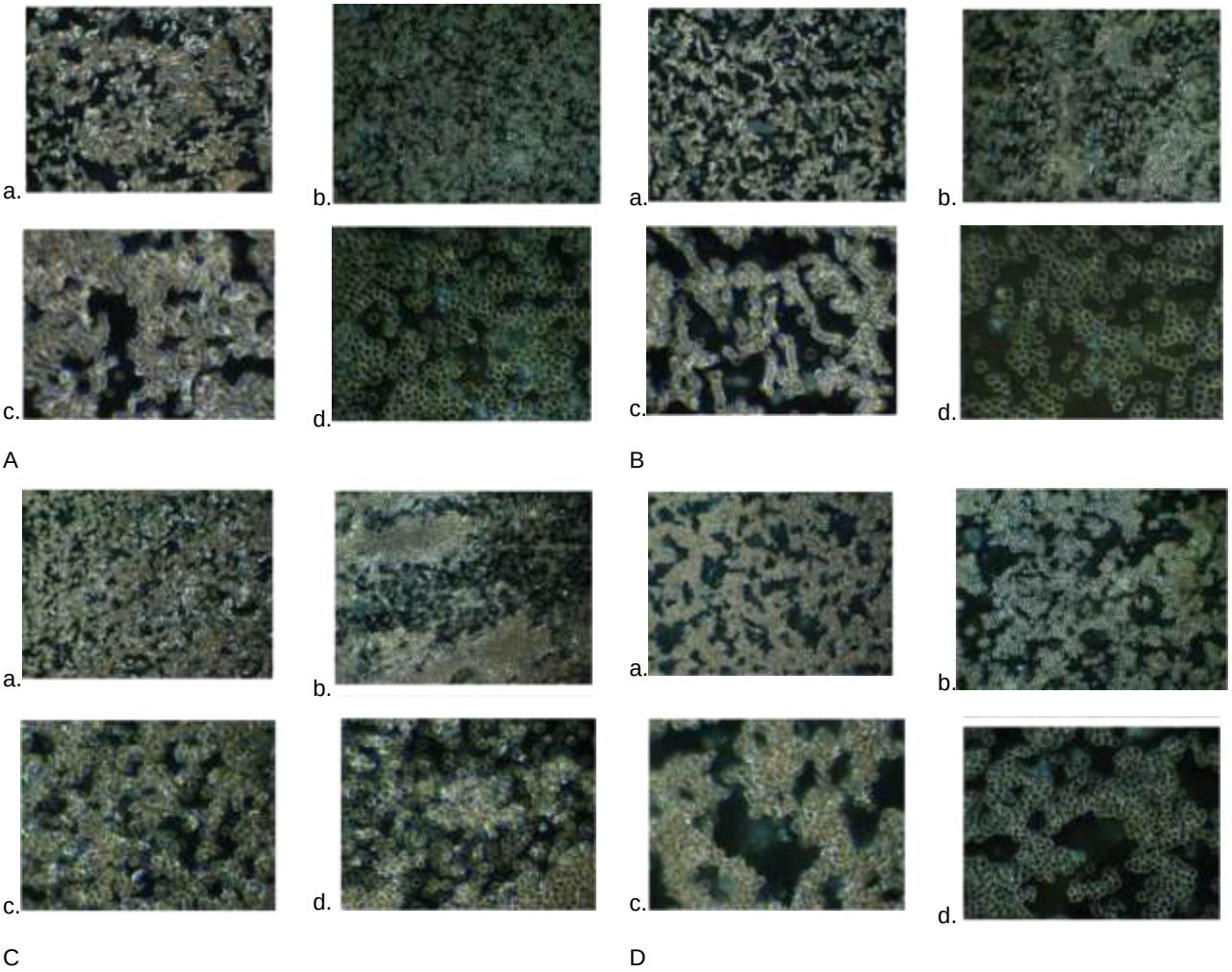

Figure 5. Mice identifications: A=2, B=3, C=6, D=7. Disaggregation of the RP in a HT29 xenograft before (a. & b.) and after (c. & d.) treatment, 100x (a & c.) and 400x (b. & d.) magnifications

One mouse (mouse no.4.) was inoculated with a PC3 human prostate tumor (from bone metastasis) cell-line; it also showed disaggregation, (see Figure 6).

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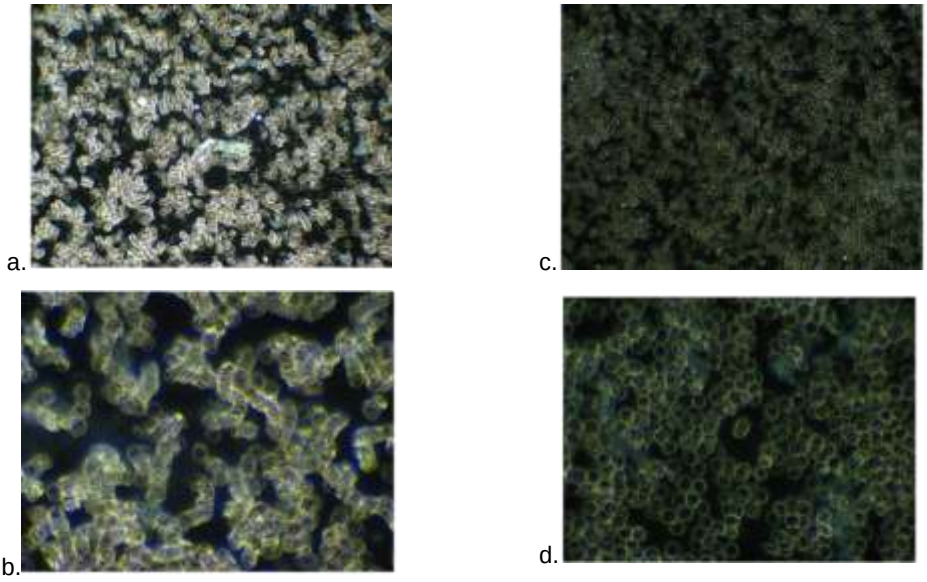
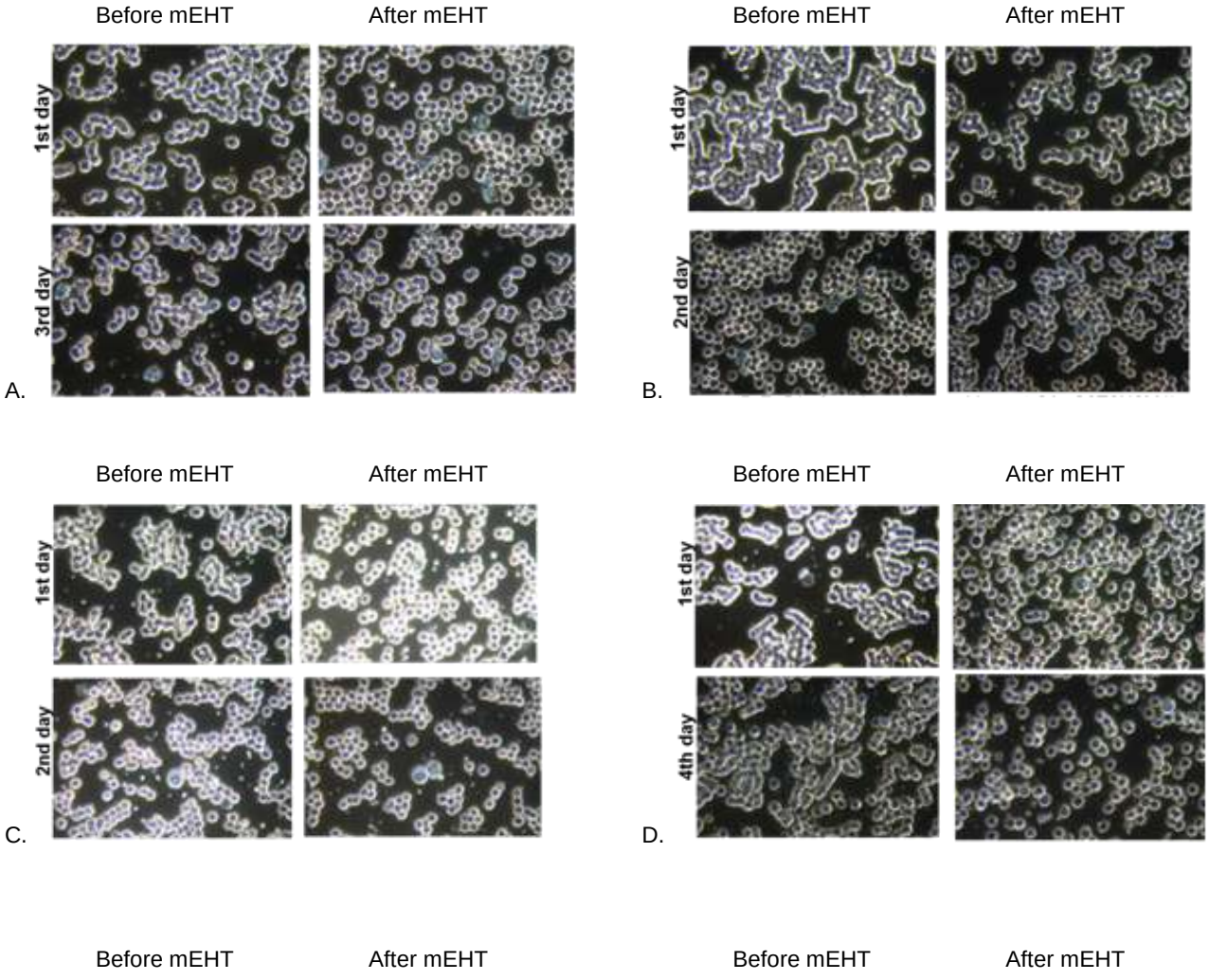


Figure 6. Disaggregation of the RP in a PC3 xenograft before (a. & b.) and after (c. & d.) treatment, 100x (a & c.) and 400x (b. & d.) magnifications

We observed the systematic distortion of RP formations in various human cancer cases, (see Figure. 7).



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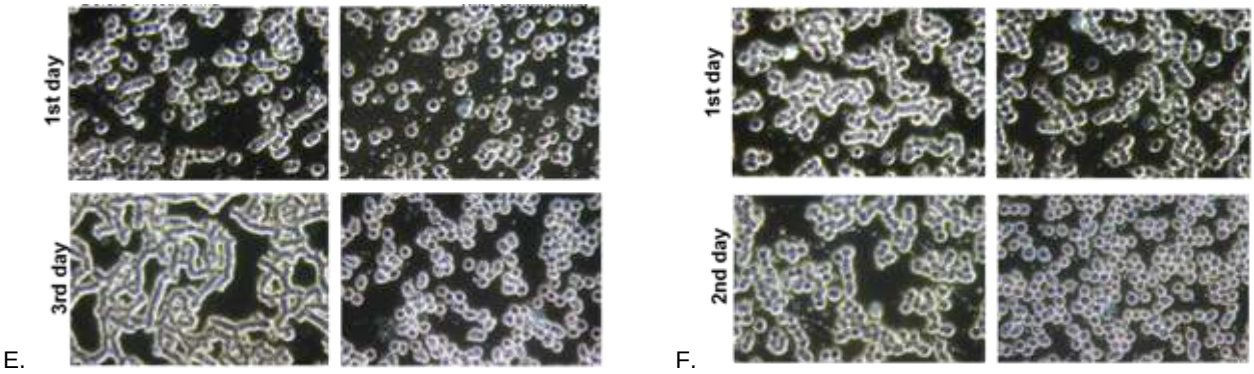

E. F.

Figure 7. Blood samples of patients with various advanced cancer lesions. A=46 y old female, advanced metastatic uterus carcinoma; B=54 y old female, locally advanced breast cancer; C=20 y old female, advanced metastatic ovarian cancer; D=61 y old female, advanced metastatic breast cancer; E=70 y old female, advanced non-Hodgkin lymphoma; F=49 y old female, advanced metastatic breast cancer.

DISCUSSION

The movement of an object caused by a spatially non-uniform electric field is the phenomena of dielectrophoresis (DEP), operating by the well-known dielectrophoretic forces created outside the electric field (it differs from the interaction of non-uniform electric fields with dielectric objects, which are suspended and free to move in a conductive medium.) In inhomogeneous alternating electric fields, the time averaged force < > that acts on a homogeneous dielectric particle can be expressed by the real part of the average of the product of the induced dipole moment and the gradient of the complex conjugate of the external field [23]. Based on this model calculation, the dielectrophoretic force from the external radiofrequency current grows by the number of erythrocytes in the RP line, (see Figure 8).

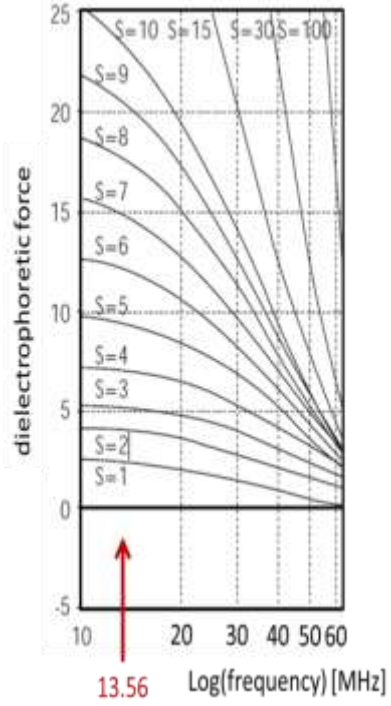

Figure 8. S=number of the erythrocytes in the RP (Modified after [23])

Keeping the RP stable, the adherent internal forces in the RP compensate for the external forces, which are measured by the use of atomic force microscopy based on single cell force spectroscopy (SCFS) [24].

The distortion of the erythrocyte aggregates could be well explained by the action of the dielectrophoretic forces when they exceed the internal adhesion. The RP are dielectric particles in an aqueous electrolyte. The inhomogeneous field polarises them together with their host matrix, the electrolyte. The polarisation creates different charges at the ends of the RP-chain, as well as in the electrolyte (see Figure 9).

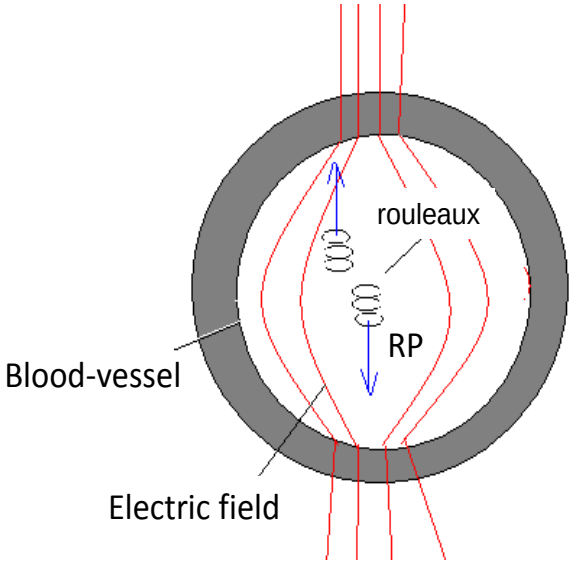

Figure 9. Effect of the outside electric field inside the blood vessel

The movement of the RP depends on the charge values at its ends and in the host electrolyte. The force depends on the dielectric properties of the RP, which is also sharply frequency dependent [25]. The relative dielectric permittivity in low frequencies (10 kHz region) could be as high as 10,000 in stationary flow; however, in turbulent cases it is only half of this value. In high frequencies this value drops to around 100 and does not affect the stationary and turbulent flow. The conductivity behaves in the opposite manner, small (~3 mS/cm) at low frequencies (~10 kHz) and in higher RF (such as 13.56 MHz, which was used in our study) it increases to 8 mS/cm. The good conductivity allows longer RF current flow along the vessel tubing. The inhomogeneous field polarises it together with its host matrix, the electrolyte. The polarisation creates different charges at the ends of the RP, as well as in the electrolyte, (see Figure 10). The movement of the RP depends on the charge values at its ends and in the host electrolyte.

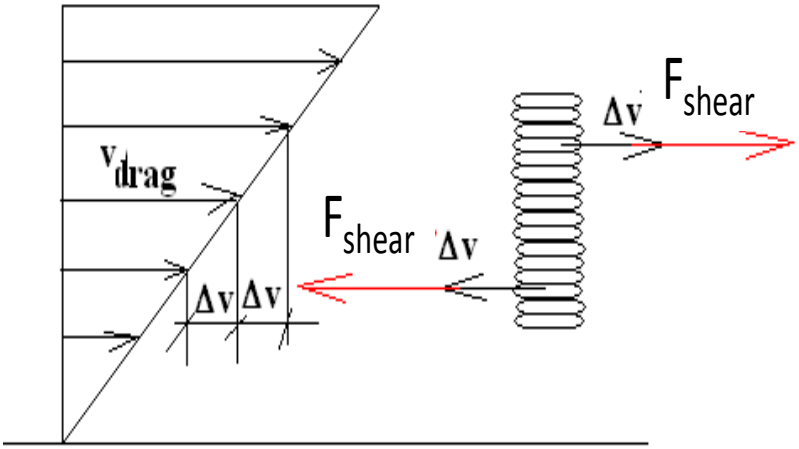

Figure 10. The shear forces, by dielectrophoretic effect, twist the RP

Dielectrophoretic force has some specialties in the 13.56 MHz region:

1. maximal polarisation exists in the axis of the RP,
2. the dielectrophoretic force grows with the length of the RP,
3. the RP fixes its direction from low filed-strength to high,
4. the maximal polarising direction in short RP is radial.

The effect of mEHT is based on the rules above. The long RP directs itself to the field-direction (rule 1.) and moves from the cork-flow to the shear flow region (rule 3.). This tendency is based on the length of the RP (rule 2.). In the region of shear-flow (Newton's flow) the middle of the RP moves with speed vdrag. Consequently, its ends have opposite drag-forces and so the shear destroys the long RP, (see Figure 11). The small parts of the destroyed RP turn perpendicularly, with their axis to the outside field, so they have no further distortions (rule 4.).

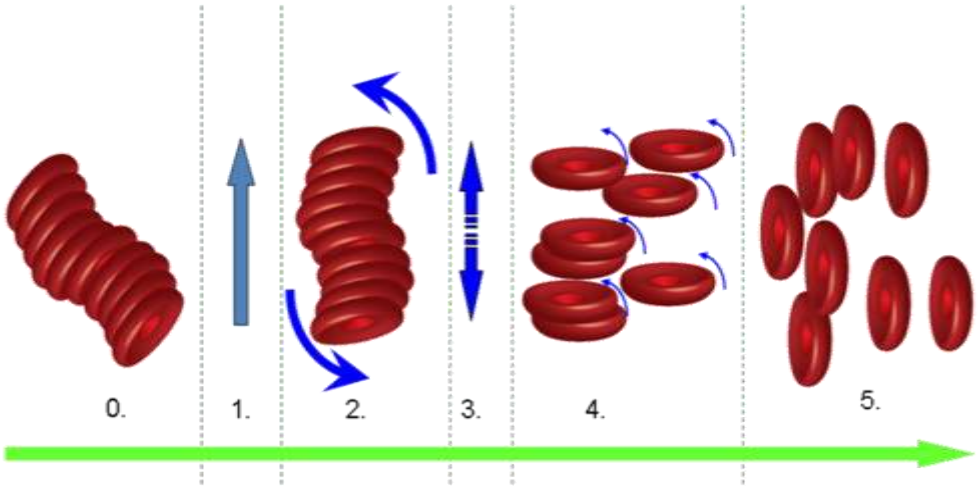

Figure 11. The RP is effectively destroyed by the gradient of EF. 0.→Original RP, 1. →EF, 2. →EF turns the RP, 3. →dielectrophoretic force breaks the adhesive connections, 4. →dielectrophoretic forces turn the erythrocytes, 5. → the aggregates of the RP are dissolved

CONCLUSIONS

In blood specimens where RP formations of erythrocytes are observed, mEHT may dissolve the aggregates. Measurement of the oncothermia effect on the RP phenomena could lead us to a simple control for treatment efficacy, but the present data cannot provide definite conclusions and further investigations are in progress.

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Author' biography with Photo

Andocs, G., DVM, PhD is a research scientist, and had been conducted scientific research work in many fields of science including radiobiology, nuclear medicine, pharmacokinetics and bioelectromagnetism. He is an author and coauthor of more than 30 papers and presently working in the Department of Radiological Sciences, Toyama University, Japan introducing the oncothermia method into the veterinary practice and conducting in vitro and in vivo basic research on radiofrequency hyperthermia method.

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