THE EFFECT OF FREQUENCY ON ELECTRIC FIELD INDUCED SURFACE FORCE IN RED BLOOD CELL MEMBRANE

Stephen KW. Chang

Biophysics and Biomedical Modeling Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA. 01760-5007

Abstract

Electric field (EF) induced surface force acting on the red blood cell (RBC) membrane has been used to explain the observed sphering and hemolysis of RBC when exposed to an EF. The frequency of the applied sinusoidal EF inversely affects the field strengths at which sphering and hemolysis occur. Increase in frequency decreases the magnitude of the surface force. The effect appears to be the result of an α-dispersion of the dielectric property of the RBC membrane.

Introduction

When an external electric field (EF) was applied to red blood cells (RBC), the RBC were observed to change from the flaccid biconcave shape to a spheroidal state. This sphering action is different from swelling in that there is no change in the RBC membrane surface area. Sphering may or may not subsequently lead to hemolysis depending on the EF strength and the time of exposure. The transmembrane potential (ΔVm) was found to be an order of magnitude lower than the accepted threshold of 1 volt [1] necessary to cause RBC membrane breakdown and lead to hemolysis. EF induced surface traction (surface force density, Ss) acting on the RBC membrane was derived using the Maxwell's stress tensor [2] and was used to explain the observed sphering and hemolysis of RBC [3].

Table 1 summarizes the experimental observations and the radial surface tractions obtained at an applied EF frequency of 1 kHz [3]. Surface traction, Ss, being always outward pointing, forced the flaccid RBC membrane outward to give the sphering action at E, = 60 V/cm. The membrane was not stretched. When EF persisted, Ss eventually stretched the membrane led to pore formation, water influx and hemolysis (at E, = 120 V/cm). At E, = 140 V/cm, Ss (at 506 dynes/cm²) was sufficiently large to tear the RBC membrane apart, hemolysis resulted quickly. This study examines the effect of the applied frequency. If E, is maintained constant, but the frequency is increased, do sphering and hemolysis still occur at the same field strength levels?

Table 1 1 kHz effects (10 dynes/cm² = 1 N/m²)

| E, (V/cm) | Experimental Observations | Ss (dynes/cm²) |
|-----------|---------------------------|---------------|
| 140       | RBC hemolyzed quickly     | 506           |
| 120       | RBC sphering, hemolysis after sustained exposure of 5-10 minutes | 372           |
| 60        | Sphering of RBC may be maintained for long period without hemolysis | 93            |
| 40        | No effect                 | 41            |

Method

The EF was generated between a pair of parallel platinum-iridium electrodes (0.5 microns in diameter) set 0.5 millimeter apart on a microscope glass slide. Blood sample was obtained by venipuncture, diluted with saline to give a 2% hematocrit solution. The 2% solution was placed between the parallel electrodes for EF irradiation. The sinusoidal EF was applied continuously. Sphering and hemolysis was observed visually under a microscope.

Results

Figure 1 shows that the threshold of sphering (E,th) increases as a function of applied EF frequency. The frequency axis (x-axis) is logarithmic, and the E, axis (y-axis) is linear. The curve displays a sigmoidal shape.

Table 2 Frequency effect on Ss

| E, (V/cm) | EF @ freq | Experimental Observation | Ss (dynes/cm²) |
|-----------|-----------|--------------------------|---------------|
| 120       | 1 kHz     | RBC sphering, slowly leading to hemolysis | 372           |
| 140       | 1 kHz     | RBC hemolyzed quickly    | 506           |
| 140       | 5 kHz     | sphering, no hemolysis   | 366           |

U.S. Government work not protected by U.S. Copyright 86
Table 2 shows the result when E, was maintained at 140 V/cm, but the frequency was increased from 1 kHz to 5 kHz. Whereas hemolysis occurred quickly at 1 kHz, RBC only sphered but did not hemolyze at 5 kHz.

Discussion
The sigmoidal shaped data curve in Figure 1 is characteristic of a dielectric dispersive behavior. However, this frequency dependency appears more like an α-dispersion than the much better known Maxwell-Wagner β-dispersion [4]. The applied frequency range (100 Hz - 10 kHz) is much lower than where β-dispersion of erythrocyte suspension resides. The existence of an α-dispersion for RBC has been described. Schwan and Carstensen [4,5] reported a characteristic frequency at 1.7 kHz for red cell ghosts and at 1.9 kHz for lysed RBC. These authors also noted that the observed α-dispersion must reflect a change in the dielectric property of the erythrocyte membrane. The characteristic frequency of this α-dispersion is at 1 kHz to 2 kHz.

The data in Figure 1 not only confirm a dispersive effect but also indicate, most likely, only a single dispersion time constant, i.e. a single characteristic frequency, is involved. The smoothness of the data curve precludes the existence of multiple time constants.

To examine how the α-dispersion of dielectric property affects the surface traction, S, one must start with Maxwell's stress tensor [6,7]

\[ T_{mn} = \Re \{ \varepsilon^* E_m E_n^* - \frac{\varepsilon^*}{2} \delta_{mn} E_k E_k^* \} \]  

\[ \varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + j\omega \tau} \]  

Equation (2) separates into

\[ \varepsilon = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{1 + (\omega \tau)^2} \]  

\[ \sigma = \sigma_\infty + \frac{(\omega \tau)^2}{1 + (\omega \tau)^2} \]  

\[ \varepsilon = \varepsilon_\infty + \frac{\Delta \varepsilon}{1 + (f/f_c)^2} \]  

\[ \sigma = \sigma_\infty + \Delta \sigma \frac{(f/f_c)^2}{1 + (f/f_c)^2} \]  

The typical parameters for RBC membrane are:

- \( \varepsilon_\infty = 11 \)
- \( \sigma_\infty = 1.0 \times 10^8 \) S/cm
- \( \Delta \varepsilon = 7 \)
- \( \Delta \sigma = 6.6 \times 10^8 \) S/cm

\( (\varepsilon_\infty \text{ and } \Delta \varepsilon \text{ are relative to } \varepsilon_\infty = 8.85 \times 10^{10} \text{ F/cm}) \)

Incorporate the typical values of Equation (5) into Equations (4) and (2) and choose \( f_c = 1.7 \) kHz, the frequency effect on surface traction, \( S \), was computed and included in Table 2. Note in Table 2, with \( E_0 \) at 140 V/cm and 1 kHz, \( S \) was 506 dynes/cm². When the frequency was increased to 5 kHz, \( E_0 \) at 140 V/cm only yielded an \( S \) of 366 dynes/cm².

The value of 366 dynes/cm² is within the range of expected values. It was expected that the \( S \) (at 140 V/cm and 5 kHz) should be comparable to 372 dynes/cm². At 372 dynes/cm², from \( E_0 = 120 \) V/cm and \( f = 1 \) kHz (see Table 1), RBC sphered and eventually hemolyzed after a sustained exposure to EF. The observation at 140 V/cm and 5 kHz was spharing but no hemolysis. Hence, \( S \) at 5 kHz should be, at maximum, comparable to 372 dynes/cm², and certainly much smaller than 506 dynes/cm², in order to explain the observation that RBC sphered but did not hemolyze.

The applied frequency of the EF has a significant effect on the RBC membrane. Frequency inversely affects the magnitude of the membrane surface traction, \( S \). Thus, increase in EF frequency delays or even prevents RBC from sphering and/or hemolysis. The frequency dependency can best be described as the result of an α-dispersion of the dielectric property of the RBC membrane.

References
1. K. Kinosita Jr., T.Y. Tsong, "Voltage-induced pore formation and hemolysis of human erythrocytes", Bioch. et Bioph. Acta, vol. 471, pp. 227-242, 1977.
2. S.KW. Chang, "Electric field induced force within the red blood cell membrane", Proc. 18th Northeast Bioengineering Conference, March, 1992. (in press)
3. S. Chang, S. Takahashi, and T. Asakura, "Volume and shape change of human erythrocytes induced by electrical fields", J. of Bioelectricity, vol. 4, no. 2, pp. 315-318, 1985.
4. H.P. Schwan, "Electrical properties of blood and its constituents: alternating current spectroscopy", Biofis, vol. 46, pp. 185-197, 1983.
5. H.P. Schwan, and E.L. Carstensen, "Dielectric properties of the membrane of lysed erythrocytes", Science, vol. 125, No. 3255, pp. 985-986, 1957.
6. H.H. Woodson and J.R. Melcher, Electromechanical Dynamics, Part II: Fields, Forces, and Motion, J. Wiley & Sons, 1968, ch. 8, pp. 418-478.
7. P. Debye, Polar Molecules. New York: Dover Publications, 1945, Chapters V and VI.

Acknowledgment
The experiment portion of this work was done at the Department of Bioengineering, University of Pennsylvania. The author is grateful to Dr. S. Takahashi of the University of Pennsylvania for his support.