ELECTRICAL TIME CONSTANT OF ERYTHROCYTE

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Abstract: The physical properties of tissues are of practical interest in medical engineering and various fields of medicine.
In this paper, the time constants of living cells were discussed. The structural relaxation (β dispersion) phenomenon is used to measure the time constant of tissues. β dispersion is often called as structural relaxation because the main cause is the cellular structure of tissues. Therefore, the time constant of β dispersion is strongly affected by the shape, membrane thickness and conductivity of intracellular fluid. It is expected that the thickness of membrane, shape and orientation of cells and conductivity of intracellular fluid can be estimated by the time constant of method. In this paper, the time constant of β dispersion. blood is mainly discussed because the blood is suitable for theoretical and experimental investigation. Blood is also very important for the physical properties of biological tissues. For example, the electrical impedance of blood is much less and the absorption coefficient of blood for visible and infrared light is much larger than that of all other living tissues. Therefore、the physical properties of blood affects much on the physical properties of biological tissues.

Introduction

In spite of the excellent theoretical and experimental work of many researchers, because of various kinds of problems we do not yet have any impedance method which is widely and reliably used in the clinical field. The estimation of measurement error is the most critical problem.

The physical properties of tissues are of practical interest in medical engineering and various fields of medicine. In this paper, the time constants of living cells were discussed [5][6][7]. Living tissues show three kinds of frequency dispersions caused by α, βand γ relaxation mechanisms [1][2]. β dispersion is often called as structural relaxation because the main cause is the cellular structure of tissues. Therefore, the time constant of β dispersion is strongly affected by the shape, membrane thickness, conductivity of intracellular fluid and orientation direction.

Therefore, we can get many informations about biological cells from β dispersion phenomenon.

We have presented a paper on the Frequency characteristics of electrical properties of living tissues and its clinical applications [14]. In these papers , the estimation error of intra- and extracellular volumes were mainly discussed. In this paper, β dispersion time constant is analytically and numerically investigated mainly for blood. The structural relaxation phenomenon (β dispersion) can be used to measure the intra- and extra-cellular fluid volumes separately [3].

The cell membrane is very thin, therefore, the capacitance of cell membrane is very large. The membrane capacitance of usual cell is about 1 μ F/cm^2, that of muscle cell is 10 μ F/cm^2 because of the tubular structure of muscle cells [1]. The membrane of biological cell has also very small conductivity compared with those of the extra- and intra-cellular fluids. When the applied electrical frequency is much lower than the β dispersion frequency, the current only flows through the extra-cellular fluid because of the membran impedance is very high, therefore the admittance of extracellular fluid Ge＝ 1 /Re is small. When the frequency is much higher than the β dispersion frequency, the current can flow through the cells because the impedanse of membrane becomes very small compare with the impedance of fluids, and the admittance Gt＝(1 /Re)+(1/Ri) becomes large. From these facts, it is expected that the extra-cellular fluid volume can be estimated by the admittance Ge and the intra-cellular volume (Gt-Ge)=Gi=1/Ri[3]. However, Ge is affected not only by the extra-cellular volume but also by the shape and the concentration of cells [12]. The orientation and deformation of cells also affect Ge and Gi [11]. Therefore, measured Ge and Gi do not show exact extra- and intra-cellular fluid volume [3][14]. Measured Ge is sometimes quite diferent from real product of extra-cellular volume and conductivity. Measured Gi also shows sometimes very large error. These effects on the measurement error are theoretically and experimetally discussed [10][14]. It is expected that the thickness of membrane, shape of cell and conductivity of intracellular fluid can be estimated from measured time constant. In this paper, the time constant of blood is mainly discussed because the blood is suitable for theoretical and experimental discussion and is also very important for the physical properties of

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biological tissues. For example, the electrical impedance of blood is much less and the absorption coefficient of blood for visible and infrared light is much larger than that of all other living tissues. Therefore, the physica properties of blood affects much on the various physical properties of biological tissues.
And it is also very important to know that the physical properties of blood remarkably change with the rate of flow.[8][11] These results contribute much not only to biorheology, but also the various field of biomedical engineering.

Materials and Methods

Electrical properties of the suspension of biological cells were theoretically discussed by many researchers [1][2][9].
For analytical calculation of electrical properties, erythrocyte is simulated by a confocal oblate spheroid shown in Figure 1. Intracellular fluid is surrounded by a membrane. Outer surface of intracellular fluid and outer surface of membrane are confocal, therefore, the thickness of membrane δ is not constant but depends on the part of erythrocytes.

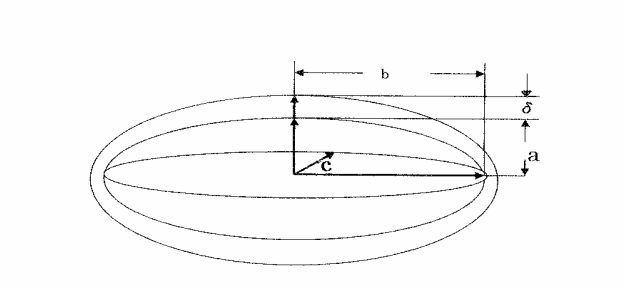

Figure1: Oblate spheroid model for an erythrocyte.
Where, a: short axis, b=c: long axis

When the current applied to a confocal spheroid parallel to one of the three rectangular axes of spheroid, the current flow through confocal spheroid has the same time constant, not depend on the part of spheroid [9]. This means that an oblate spheroid has only two time constant because the two axes in three rectangular axes has the same time constant. .

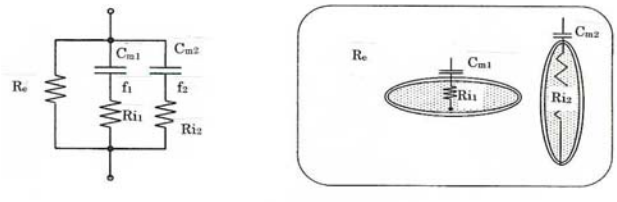

Figure 2: Oblate Spheroid Model of Erythrocytes orient themselves perpendicular each other, and equivalent circuit of two time constants.

For real erythrocytes in blood, size and orientation direction are not the same but distributed. Therefore, their time constant is also distributed. In this paper, time constant distribution is not discussed because all erythrocytes are supposed to orient same direction .
The conductance of particle suspension can be theoretically calculated under some assumptions by Fricke's Equation (1)

$$\sigma_i = \sigma_s + \frac{\rho}{1-\rho} \sum^{j=x,y,z} \frac{2k_j (\sigma_{pj} - \sigma_i)}{2 + abcL_{ij}\left[(\sigma_{pj}/\sigma_s) - 1\right]} \quad (1)$$

Where σ_i : conductivity of cell suspension when current flows i direction, σ_s: conductivity of extra-cellular fluid (plasma), ρ : Hematocrit, σ_{pj}: admittance of particle oriented j direction, *Kj*: fraction of erythrocytes orient themselves j direction. L_{ij}: shape factor of spheroid oriented j direction when current flows i direction. a,b,c are the length of three rectangular axis.
Another equation can also be used for this calculation.[12][14]. Physical meaning is more clear than Fricke's equation. Here, the equation is briefly shown.
It is rather difficult to understand the physical meaning of this Fricke's equaation.. The same equation can easily be derived by compensation theorem for continuous media, and as shown below.

$$\sigma_i = \left[1 - \rho\left(\sum_{j=a,b,c}(1+\varepsilon_{ij})K_j\right)\right]\sigma_s + \rho\left(\sum_{j=a,b,c}(1+\varepsilon_{ij})K_j\sigma_{pij}\right) \quad (2)$$

In this equation, the conductivity of blood is considered to be the parallel summation of four components such as plasma and the three components of the erythrocytes oriented to one of three radii parallel to measurement axis i. Equation (2) can also easily be derived from equation (1) if the assumptions are satisfied. Where $(1+\varepsilon_{ij})$ shows the effect of erythrocytes on the effective volume of plasma. Therefore, the apparent (measurement) hematocrit value becomes $(1+\varepsilon_{ij})\rho$. ε_{ij} are the relative differences between apparent and real volume fractions for erythrocytes oriented j directions. Therefore, ε_{ij} shows the relative measurement error of intra-cellular fluid volumes derived from compensation theorem. What we want to know is the product of fluid volume fraction and conductivity for both intra- and extra-cellular fluids. The real product of extra-cellular fluid fraction and conductivity is $(1-\rho)\sigma_s$ and that of intra-cellular fluid is $\rho\,\sigma_p$. When all erythrocytes are oriented in the j direction, relative ratio of apparent and real products for extra- cellular fluid Koej = σ io/{(1-ρ)σs) and for intra-cellular fluid Koij=(σi_{∞} - σ io)/{ $\rho\,\sigma_p$ }can be derived from equation(2) for various hematocrit values and various axis ratios. σ io and σi_{∞} are the conductivities at 0Hz, the lowest measurement frequency and at ∞Hz, the highest. The measurement results are agree well with the results calculated by equation (2). When the current flows in the x direction (i=x), the relative ratio of measured and real conductivity Koej and Koij can be

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easily be calculated by equation (3). . Hereafter, when the short axis a of the oblate spheroid model is parallel to the i direction, the orientation direction is shown as j = x. When the long axis b or c is parallel to the i direction, j=y or j=z. And for random orientation, j=r.

$$1+\varepsilon_j = \frac{\dfrac{2k_j}{2+abcL_j\left((\sigma_{pj}/\sigma_s)-1\right)}}{1-\rho+\rho\left\{\dfrac{2k_x}{2+abcL_x\left((\sigma_{px}/\sigma_s)-1\right)}+\dfrac{2(k_y+k_z)}{2+abcL_y\left((\sigma_{py}/\sigma_s)-1\right)}\right\}} \quad (3)$$

Where, ε_j is the measurement error when j radius of all oblate spheroidal particles orient themselves parallel to the measurement direction i. The effects of cells oriented in the b or c directions are the same. Equation (3) reduces to equation (4) when current flows in the same direction.

$$1+\varepsilon_x = \frac{1}{\dfrac{(1-\rho)\left(2+dbcL_x\left((\sigma_{px}/\sigma_s)-1\right)\right)}{2k_x}+\rho\left\{1+\dfrac{k_y+k_z}{k_x}\dfrac{\left(2+dbcL_x\left((\sigma_{px}/\sigma_s)-1\right)\right)}{\left(2+dbcL_y\left((\sigma_{py}/\sigma_s)-1\right)\right)}\right\}} \quad (4)$$

$$1+\varepsilon_{y+z} = \frac{1}{\dfrac{(1-\rho)\left(2+dbcL_y\left((\sigma_{py}/\sigma_s)-1\right)\right)}{2k_y}+\rho\left\{1+\dfrac{k_x}{k_y+k_z}\dfrac{\left(2+dbcL_y\left((\sigma_{py}/\sigma_s)-1\right)\right)}{\left(2+dbcL_x\left((\sigma_{px}/\sigma_s)-1\right)\right)}\right\}}$$

From these equations. β dispersion frequency for j direction can be obtained.

$$f(\beta)_j = \frac{1}{2\pi C_{bmj} r_{dj}} = \frac{1}{2\pi\left[\dfrac{C_{mj}(1-\rho)abcL_j}{\sigma_s\left(2-(1-\rho)abcL_j\right)}+C_{mj}r_{pj}\right]}$$

$$= \frac{1}{2\pi C_{mj}\left(r_{pj}+\dfrac{(1-\rho)abcL_j}{\sigma_s\left(2-(1-\rho)abcL_j\right)}\right)}$$

Where j is the rectangular orientation direction parallel to the current direction i. C_{mj} and r_{pj} is the membrane capacitance and resistance of cell in j direction per 1cm^2. Therefore, C_{bmj} and r_{dj} are the effective membrane capacitance and effective resistance of intra-cellular fluid per 1 cm^2. $C_{bmj} \cdot R_{dj}$ is the β dispersion time constant.

If the all erythrocytes are orient themselves in the same direction and an axis of spheroids coincides the current direction, time constant of the suspension is single, When an erythrocyte orients itself to an arbitral direction, we can divide it into two erythrocytes, one part orient itself as the current is parallel to an axis and another part orient b axis direction. Therefore, spheroid suspension has two time constants as shown in Figure2.

However, real shape of an erythrocyte is circular disk with biconcave cross section as shown in Figure 3.

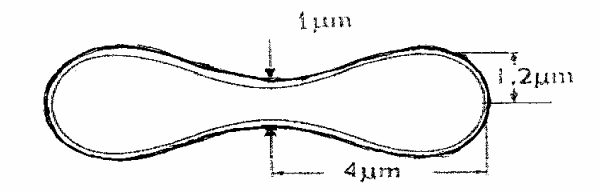

Figure 3: A model of erythrocyte with biconcave cross section.

This model can not be discussed analytically.

In this paper, boundary element method (BEM) is used for the numerical calculation of time constants for this realistic model.

From above explanations, it is easy to understand that the time constant of blood is not single but distributed. The membrane capacitance C_{bmj} is the same at all over the membrane but intracellular fluid resistance R_{dj} depends on the part of a cell, therefore, the time constant $C_{bmj} \cdot R_{dj}$ must be distributed. This phenomenon occurs even when all erythrocytes orient themselves to the same direction. And the sizes of erythrocytes are the same.

Results

For check our program of BEM numerical calculation, at first sphere suspension was calculated. Numerical calculation results must be quite well agreed with the analytically calculated results. For sphere, oblate spheroid model is the same with biconcave model.

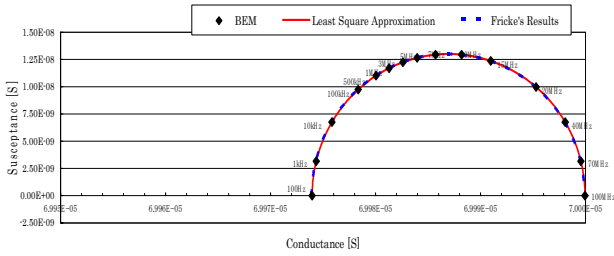

Figure 4: Admittances locuses of sphere suspension calculated by numerical (biconcave) and analytical (oblate spheroid) method. Numerical calculation results (red) are quite well agreed with analytically calculated results (blue).

The comparison of Numerical (BEM) and analytical (Fricke's Method) calculated results for the same sphere.

Single sample sphere is suspended at the centre of the 50 μ m X 50 μ m X 50 μ m cubic extra-cellular fluid.

Intra-cellular fluid conductance σ_1: 0.8X10^{-6} S/ μ m .

extra-cellular fluid conductance σ_1: 1.4X10^{-6} S/ μ m

membrane capacitance of erythrocyte: 1 μ F/cm^2

diameter of sphere: 6 μ m

relaxation frequency f(β): 3MHz

The shape of suspended erythrocyte for analytical calculation is simulated confocal oblate spheroid shown in Figure1.

The shape of suspended erythrocyte for numerical calculation is simulated biconcave circular disc shown in Figure2,

From these results the numerical calculation method (BEM) is verified to calculate accurate results.

ISSN: 1727-1983

Some of our results calculated by theoretical equations both analytical (oblate spheroid) and numerical (biconcave) methods are shown in Figure 5 and Figure6. In Figure 5, frequency characteristics of oblate spheroid model calculated by analytical method for three case (transverse, parallel, and random oriented) are shown.
At very low frequencies, conductance is rather small and susceptance is also very small. Conductance and susceptance increase with the increase of frequency. Conductance approaches a constant value. Susceptance increases to maximum value and then decreases. This phenomenon shows typical β dispersion. Frequencies at maximum susceptance are called β dispersion frequency f(β). 1/f(β) is β time constant.

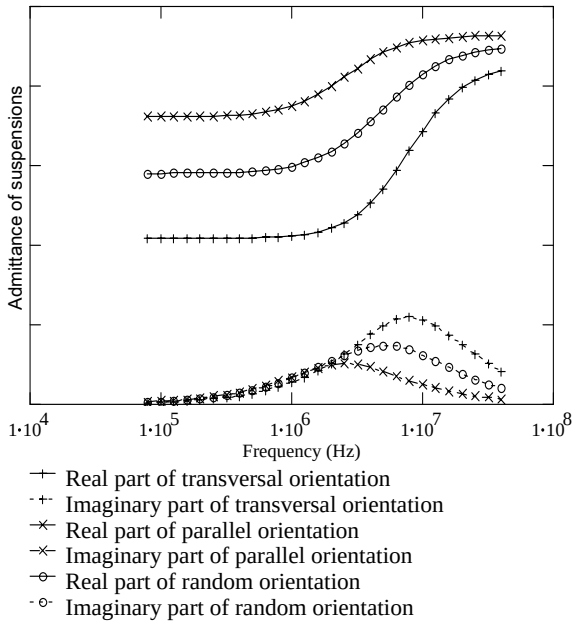

Figure 5: Frequency characteristics of a blood

When the short axis of all erythrocytes (spheroids) orient themselves parallel to the current direction (transversal orientation), conductance(real part of admittance) is quite low and f(β) is very high. When the long axis of all spheroids orient themselves to the current direction (parallel orientation), conductance is very high and f(β) is very low.
When the spheroids orient themselves at random(random orientation), both conductance and f(β) are between transversal and parallel orientation.
The measured time constant for parallel orientation blood is highest and about 8MHz.
That for transverse orientation blood is lowest and about 2.5MHz.
That for random orientation blood is between them and about 5MHz.

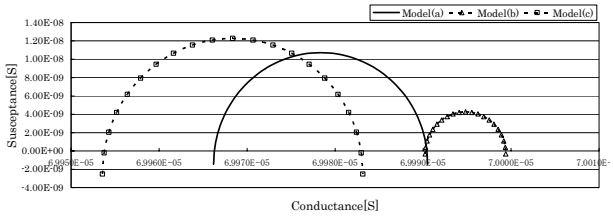

Figure 6: Admittance locuses calculated by analytical method (Fricke's equation). As shown above, analytical method has two time constants for transverse and parallel orientations shown in Fig. 2. And the admittance locus for random oriented blood is also shown.
Two semicircles are corresponding to transversal (left) and parallel orientation (right). The other semicircle in centre is corresponding to random orientation.

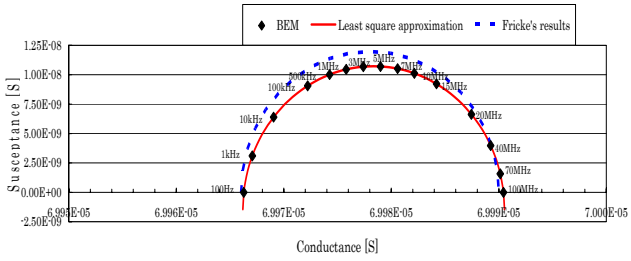

Figure 7: Admittances locuses of parallel oriented blood calculated by numerical (biconcave) and analytical (oblate spheroid) methods. The shape of admittance obtained by analytical method is a semicircle shown by blue line. From analytical results it looks like that the erythrocytes has only one time constant. However, the shapes of the results obtained by numerical calculation are not a semicircle (arc) with the centre below real axis. This means that the erythrocytes have distributed time constant. The main cause of this difference is the difference of models. Analytical model is oblate spheroid shape. This shape has theoretically single time constant. This shape derived that the erythrocyte does not have multi time constants, but single time constant for easy calculation.
But as I explained above, numerical model is quite realistic because the shape is biconcave and membrane thickness is the same anywhere. Therefore, it has distributed time constant. The shape and size of erythrocytes are little distributed. But it affects not so big.

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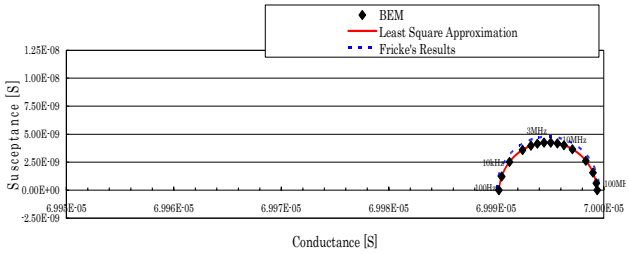

Figure 8: Admittances locuses of transverse oriented blood calculated by numerical (biconcave) and analytical (oblate spheroid) methods. Results are the same as parallel orientation. The shape of admittance obtained by analytical method is also a semicircle. And the results obtained by numerical calculation are also arc of a circle with the centre below real axis. The main cause is also
the same. The effects are much larger than parallel orientation.

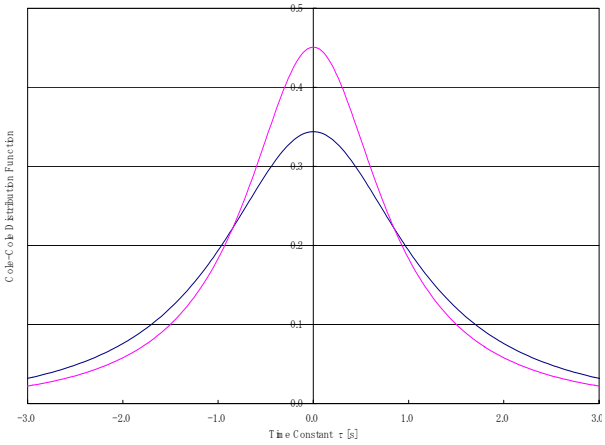

Figure 9: Cole distribution function of β dispersion time constant for blood calculated by numerical method. Time constant distribution for parallel orientation (blue line) is smaller than the time constant distribution for transverse orientation (red line).

This method also applies to the time constant measurement for muscle. Muscle fibre can also be simulated by prolate spheroid and numerical model. In this paper the length of muscle fibre is five times larger than the radius at the centre of muscle. Radiuses are 10 μ m in length and 2 μ m in diameter.
In this paper, all calculation are done under the assumption that single muscle fiber is suspended in 50 μ m x 50 μ m x 50 μ m interstitial fluid.

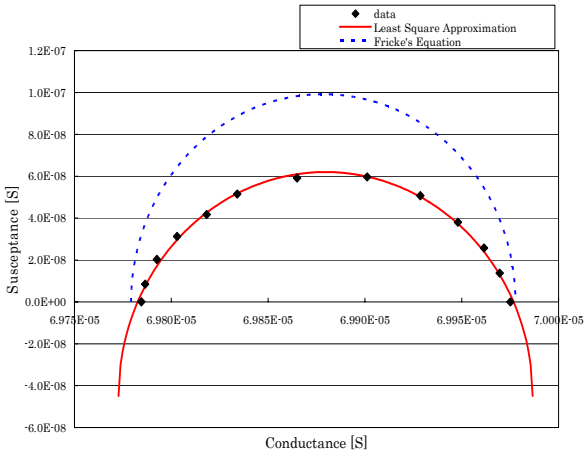

Figure 10: Admittance locus for the transverse oriented Muscle fibre. Fricke's model (blue line) also shows semicircular admittance locus, but resu;ts calculated BEM method also show the time constant distributed locus. The cause of this phenomenon is also the same as blood. But distribution is much larger than blood.

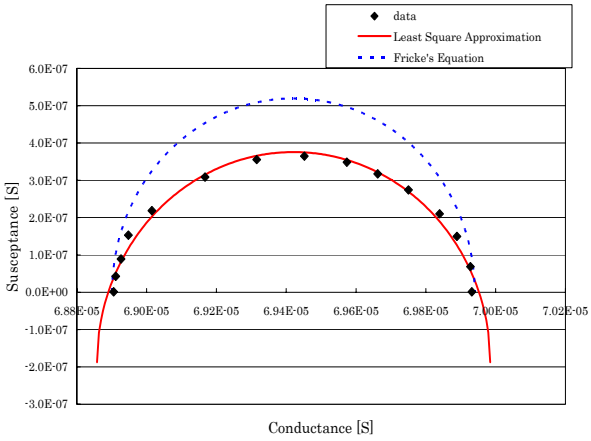

Figure 11: Admittance locus for the parallel oriented Muscle fibre. Fricke's model (blue line) also shows semicircular admittance locus, but results calculated BEM method also shows the time constant distributed locus. The cause of this phenomenon is also the same as blood. But distribution is much larger than blood.
For blood time constant distribution in transverse direction is much larger than Parallel direction. For muscle, parallel oriented fiber shows much higher distribution than tranverse direction fiber. distribution much larger

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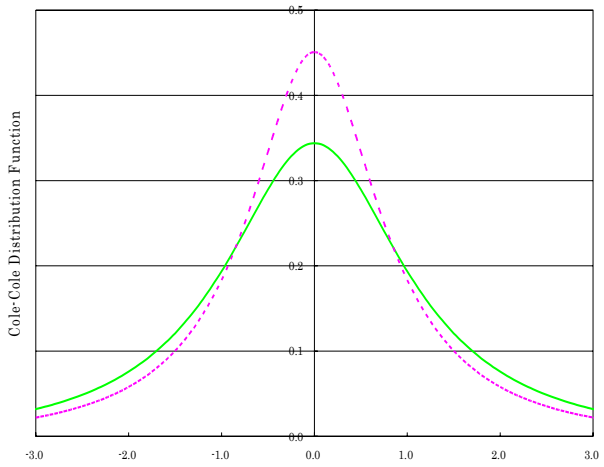

Figure 9: Cole distribution function of β dispersion time constant for muscle fibre is calculated by numerical method. Time constant distribution for parallel orientation (green line) is smaller than the time constant distribution for transverse orientation (pink line).

Discussion

Electrical time constant of biological tissues are sometimes very useful to investigate physical properties. In this paper, the β dispersion frequency is applied to investigate the physical properties of cellular structure. Blood and muscle fibre are mainly investigated.

β dispersion phenomenon is structural relaxation. In this paper relaxation caused by cellular structure are discussed. To analyze cellular structure, electrical measurement is one of the very useful tool. From electrical properties, we can estimate, intra-cellular fluid volume, extra-cellular volume, conductivity and hematocrit, fat volume, etc. From these measurement results, we can estimate a lot of biological information.

In this paper, we try to estimate electrical time constant of blood and muscle fibre. Electrical time constant can be measured from β dispersion frequency. Electrical time constant closely related to cell membrane, intra-cellular fluid, shape and size, orientation of cells, etc.

Admittances locuses of transverse oriented blood calculated by numerical (biconcave) and analytical (oblate spheroid) methods.

There are some differences between two method, because of the differences of the models. The difference is not so large. The main defference is the shape of admittance change. Analytical model has single time constant, but numericl model has distributed time constant. Therefore the shape of admittance locus for numerical method is arc (a part of circle with the centre under real axis). The calculated shape of admittance locus by analytical method is semicircle because of single time constant.

From the theoretical calculation the relation between the shapes of cells and physical properties are theoretically understand.

We have already many measurement results. Theoretical results obtained in this paper should be compared with the measurement results.

Conclusions

Two theoretical methods are discussed in this paper.

Frick's equation is analytical and very useful, but it is not suitable for time constant measurement. Because Fricke's model is based on spheroid model, therefore, it is very useful to calculate the centre relaxation frequency. However, it is not suitable to measure distribution of relaxation frequencies.

Numerical calculation method is useful for obtain the distribution of relaxation time constant.

Time constant include many important biological information. It is desired to develop simple calculated method for time constant distribution.

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