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Effects of rigidity on tension within the cell membranes of erythrocytes swollen by osmotic shock

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Abstract
Erythrocytes swell owing to the osmotic shock caused by hypotonic liquids, and when the membrane tension exceeds a certain limit, hemolysis occurs. The base tension in the membrane of a spherically shaped erythrocyte is usually ignored in the mechanical evaluation of hemolysis. However, the base tension cannot be ignored when the rigidity of the erythrocyte membrane increases owing to lesions, oxidative stress, and other phenomena. Therefore, it is necessary to re-evaluate the tension level at which hemolysis occurs by considering the increased base tension, which is caused by a combined increase in the bending and shear rigidity of the membrane. To achieve this, we calculated the effect of increases in the combined rigidity on the increases in the internal pressure and membrane tension of the erythrocyte. In this study, assuming the surface area to be constant, the swelling process of erythrocytes was evaluated under the condition that hemolysis does not occur. Evaluation was performed by minimizing strain energy, which is the sum of bending strain and shear strain. When the erythrocyte was spherical, the membrane base tension increased linearly with combined rigidity. Even when the bending rigidity was increased to 100 times that of normal erythrocytes, the effect of the base tension on the hemolysis tension level (15 mN/m) was negligible. However, when shear rigidity was increased to 50–100 times that of normal erythrocytes, it became necessary to decrease the hemolysis tension level by 10% and 20%, respectively, because the base tensions were approximately 1.5 and 3.0 mN/m, respectively.

Keywords: Erythrocyte, Hypotonic osmotic shock, Hemolysis, Bending rigidity, Shear rigidity, Strain energy minimization, Membrane tension

1. Introduction

When osmotic shock is applied to erythrocytes using a hypotonic solution, water infiltrates the erythrocytes causing swelling, which eventually leads to hemolysis. The hemolytic and osmotic resistance characteristics of erythrocytes can be investigated by examining the erythrocyte swelling process (Rand, et al., 1963; Canham, et al., 1970; Jay, et al., 1975). Although the shape of an erythrocyte in its natural state is a biconcave disk, during the swelling process, it expands into a sphere with a constant surface area (Evans, et al., 1972; Jay, et al., 1975). Subsequently, the radius of the sphere increases, leading to an increase in membrane tension. Hemolysis occurs when the membrane tension exceeds a certain value. The level of tension is estimated by a suction experiment of a portion of erythrocytes using a micropipette, and its corresponding theoretical analysis (Rand, 1964).

The rigidity of the erythrocyte membrane increases due to diabetes (McMillan, et al., 1983; Chang, et al., 2017), malaria (Suresh, 2006; Zhang, et al., 2015), and oxidation (Hebbel, et al., 1990; Hale, et al., 2011). Since the erythrocyte membrane comprises the lipid bilayer membrane and spectrin network, the increase in overall rigidity is a result of the combined increase in bending and shear rigidity, caused by lipid bilayer mutation and spectrin mutation, respectively. During erythrocyte swelling due to the osmotic shock, there is a higher increase in membrane tension when the membrane rigidity increases than when it does not. Therefore, the erythrocytes with increased membrane rigidity undergo hemolysis even with a minute increase in membrane area.
The tension level that leads to hemolysis is evaluated based on the moment when the erythrocyte swells to a sphere with a constant surface area. In this case, the reference tension, that is, base tension is neglected, as the pressure inside the erythrocytes is considerably less (Rand, 1964). Although this assumption is valid for normal erythrocytes, for those with increased membrane rigidity, the pressure at the moment the erythrocyte expands to a sphere is high; hence, the reference base tension may not be negligible. The reference pressure and tension are realized as the final state of the swelling process where the surface area of the erythrocyte remains constant. Therefore, to evaluate these values, it is necessary to observe the swelling process until the spherical shape is obtained. In order to analyze the swelling process, this study employs the method of minimizing strain energy that requires numerical calculation (Zarda, et al., 1977; Pai, et al., 1980; Fischer, et al., 1981). First, to examine the validity of the calculation method, we compared the results with the calculation results of the solution method of erythrocyte swelling that involves considering the differential equation as the governing equation (Pozrikidis, 2003), and with the results of an experiment that involves application of osmotic shock to the erythrocytes (Bando, et al., 2020). Next, we examined the effects of an increase in the bending and shear rigidity on the increase in internal pressure and membrane tension of the erythrocytes.

The purpose of the present study is as follows. The increase in the membrane base tension for a spherical erythrocyte with swelling was calculated for increases in shear rigidity and bending rigidity. Then, we examined the necessary decrease in the tension level at which hemolysis occurs owing to the existence of the base tension. This is because the tension level of hemolysis occurrence is usually evaluated under the assumption that the base tension is sufficiently small, and only the tension caused by surface area expansion from the spherical base state has typically been considered.

2. Analysis

Erythrocytes are assumed to be axisymmetric in shape. As shown in Fig. 1, the coordinates \((r, z)\) were taken in the meridional plane. Only the first quadrant was considered assuming symmetry boundary conditions on the \(r\)- and \(z\)-axes. The principal curvature taken from the first quadrant \(\kappa_r\) is given by the following equation:

\[
\kappa_r = \frac{\pi}{2L} \left(1 - \sum_{n=1}^{N} c_n \cos \left(\frac{(2n-1)\pi s}{L}\right)\right)
\]  

(1)

In the right side of the above equation, \(s\) is the length along the contour line of the erythrocytes, which was taken as \(s=0\) on \(z\)-axis, and \(L\) is the total length. Equation (1) is an extension of the function used to estimate the natural shape of erythrocytes (Pozrikidis, 2003). The principal curvature \(\kappa_\phi\) in the plane orthogonal to the \(s\) axis is given by the following equation:

\[
\kappa_\phi = \frac{1}{r} \sin \left[\frac{\pi}{2L} \left(s - \frac{L}{\pi} \sum_{n=1}^{N} c_n \sin \left(\frac{(2n-1)\pi s}{L}\right)\right)\right]
\]  

(2)

We used \(N = 3\) for the calculation.

The shape of the erythrocyte during the swelling process was determined in such a manner that the total strain energy caused due to the bending and shear deformation of the erythrocyte membrane was minimized. The constraint conditions are namely that the surface area of the erythrocyte remains the same as in its unstressed initial state, and the volume remains equal to the volume specified in the procedure [3] shown below.

The strain energy of bending deformation per unit area denoted by \(W_B\), and the strain energy of shear deformation per unit area denoted by \(W_S\) are given as follows (Evans, et al., 1980):

\[
W_B = \frac{D}{2} \left[(\kappa_s - \kappa_{s0})^2 + (\kappa_\phi - \kappa_{\phi0})^2\right]
\]  

(3)

\[
W_S = \frac{\mu}{4} (\lambda_s^2 + \lambda_\phi^2 - 2)
\]  

(4)

where \(D\) is the bending rigidity, \(\mu\) is the shear rigidity, and the subscript ‘0’ represents the unstressed initial state. \(\lambda_s\),
is the principal stretch ratio in the meridional plane, and \( \lambda_\phi \) is the principal stretch ratio in the circumferential direction, which are respectively given by the following equations:

\[
\lambda_s = \frac{ds}{ds_0}, \quad \lambda_\phi = \frac{r}{r_0}
\]

(5)

Note that both \( s_0 \) and \( s \), and \( r_0 \) and \( r \) indicate the coordinates at the initial stage and deformed state, for the same material points. When the surface area is assumed to be constant, the following equation holds true.

\[
\lambda_s \lambda_\phi = 1
\]

(6)

When calculating equations (3) and (4), the same material points for the initial stage and deformed state are obtained by numerically solving the following equation for \( s \).

\[
\int_0^s 2\pi r_0 ds_0 = \int_0^s 2\pi r ds
\]

(7)

The procedure followed for calculation is detailed as follows:

[1] Determined the initial shape by giving \( L, c_1, c_2, c_3 \). Determined volume \( V_0 \), and surface area \( A_0 \) corresponding to the initial shape, and volume \( V_{\text{max}} \) for when it expanded to a sphere with a constant surface area.

[2] Verified that the volume \( V \) gradually increases from \( V_0 \) to \( V_{\text{max}} \).

[3] Specified \( c_2, c_3 \) and then determined \( L, c_1 \) such that the volume became \( V \) and the surface area became \( A_0 \). The values of \( L, c_1, c_2, c_3 \), and \( c_4 \) determine the shape of the erythrocyte through the principal curvature of Eq. (1). The minimization parameters were taken as \( c_2, c_3 ; L, c_1 \) were used to satisfy the constraints for the volume and surface area by using the Newton–Raphson method.

[4] Computed the total strain energy \( E = E_B + E_S \) by integrating equations (3) and (4) over the erythrocyte surface. Here, \( E_B \) and \( E_S \) is the strain energy caused due to bending and shearing, respectively.

[5] Computed \( c_2, c_3 \) again and repeated steps [3] to [5] such that \( E \) reaches the minimum possible value. On a two-dimensional square mesh taken in the ranges \( c_2, c_3 \in [c_{2,\min}, c_{2,\max}] \) and \( c_3, c_4 \in [c_{3,\min}, c_{3,\max}] \), the values of \( c_2 \) and \( c_3 \) that minimize \( E \) were searched by point-by-point sweeping.

[6] The shape when \( E \) reaches the minimum value was taken as the shape for volume \( V \). The pressure inside the erythrocyte was determined using the following equation:

\[
p = \frac{dE}{dV}
\]

(8)

[7] Repeated steps [2] to [7] and when \( V = V_{\text{max}} \), indicating a spherical erythrocyte, calculated the membrane tension from the following Laplace equation and completed the calculation.

\[
T = \frac{1}{2} \left( \frac{3V_{\text{max}}}{4\pi} \right)^{1/3} p_{\text{max}}
\]

(9)

where \( p_{\text{max}} \) is the pressure for the spherical erythrocyte. This tension value \( T \) is taken as the base tension in evaluating the hemolysis occurrence tension, which is determined by adding the base tension to that caused by the surface area expansion ratio of spherical erythrocytes expanded from the base state.

The physical quantities associated with the erythrocyte swelling are pressure \( p \), reference length \( \ell \), bending rigidity \( D \), and shear rigidity \( \mu \). The dimensional analysis (Igarashi, et al., 2013) of these quantities results in the following dimensionless parameters:

\[
\pi_1 = \frac{p\ell^3}{D}, \quad \pi_2 = \frac{\mu\ell^2}{D}
\]

(10)

Therefore, the dimensionless quantity \( \psi \) associated with the erythrocyte shape, such as \( (V_{\text{max}} - V)/V_{\text{max}} \), can be expressed as follows:

\[
\psi = f(\pi_1, \pi_2)
\]

(11)

where \( f \) is a function of \( \pi_1 \) and \( \pi_2 \). Further, \( \pi_2 \) and \( \pi_1/\pi_2 \) are independent such that \( \psi \) can be expressed as:

\[
\psi = g(\pi_2, \pi_3)
\]

(12)
where $g$ is a function of $\pi_2$ and $\pi_3$, with $\pi_3$ being expressed as

$$\pi_3 = \frac{p_f}{\mu}$$

(13)

The parameters $\pi_1$, $\pi_2$, and $\pi_3$ were defined by Zarda, et al. (1977). The parameters in Eqs. (10) and (13) are used to analyze the change in the base tension with changes in the bending and shear rigidity.

3. Validation of Method

Figure 2 shows the relationship between the dimensionless pressure and volume of an erythrocyte as it swells. The solid line denotes the calculation results, and the dotted line denotes the results from the literature (Pozrikidis, 2003). The dotted line does not include the results for $\hat{p}=38$ or more. In Pozrikidis (2003), a differential equation was derived by balancing the force and moment of a small portion of the membrane. The result shown in Fig. 2 was obtained by solving the said equation. The natural shape of the erythrocyte was estimated by equating $\kappa = \frac{\pi}{2L}(1-\delta \cos(\pi \delta / L))$, (where $\delta = 2$) as the initial curvature. Further, assuming the shear rigidity to be zero, the bending moment was calculated from the difference in curvature between points with the same dimensionless coordinates of the initial and deformed stage, rather than from the difference in curvature between the same material points at the initial and deformed stage. Therefore, in this study, the calculations were performed based on the assumptions discussed above, and the obtained result is shown through the solid line in Fig. 2. In the figure $\hat{p}$ is defined as $\hat{p} = pa^3 / D$, where $a$ is the spherical radius corresponding to $V_{\text{max}}$. Additionally, in Fig. 2, the solid line almost coincides with the dotted line. Thus, the strain energy minimization method used for this calculation can obtain a solution equivalent to the one obtained from the differential equation method. The absence of complete overlapping of the solid line with the dotted line may be a result of the change in curvature of the erythrocyte membrane, as detailed in Eq. (1) and where we assumed $N = 3$ for this calculation.

The consistency between the strain energy minimization method and the mechanical equilibrium method was proved by Kralchevsky, et al. (1994). However, according to our numerical calculations using the mechanical equilibrium method, which was the same as that for the swelling problem in Pozrikidis (2003), a converged solution could not be obtained when the shape was nearly spherical. This may be due to the problem of stiffness, that is, when the shape is nearly spherical, small changes in the volume induce large pressure changes, as shown in Figs. 5 and 6. This problem can be avoided by using the strain energy minimization method.

![Fig. 2 Relationship between the pressure change and volume change of the swelling erythrocyte model.](image-url)
membrane was taken as $D = 1.8 \times 10^{-19}$ Nm, and the shear rigidity was taken as $\mu = 6.0 \times 10^{-6}$ N/m (Hochmuth, et al., 1987; Mohandas, et al., 1994). Further, to match the initial shape with the experimental shape at $t = 0\text{s}$, we assumed $L = 4.9 \mu m$, and $c_1 = 1.9$, $c_2 = c_3 = 0$. The erythrocyte volume and surface area of the initial shape were $V_0 = 105 \mu m^3$, and $A_0 = 144 \mu m^2$, respectively, which are within the values stated in the literature (Burton, 1972). The shapes obtained in the calculation and the experiment were compared to ensure that sphericity—described as the ratio of length of minor axis to that of major axis—matched. The erythrocytes were observed using a phase-contrast microscope, which allowed us to observe the white halos formed around the erythrocytes (Pluta, 1988). The contour of the erythrocytes, denoted by the solid red line, was nearly equivalent to the manual contour plots of the erythrocyte image obtained by Jay (1975).

![Fig. 3 Comparison of the experimental results (Bando, et al., 2020) and calculation results (red solid line) of the swelling deformation of an erythrocyte.](image)

In the experiment, hemolysis did not occur even when the erythrocyte became spherical; further, it remained in a steady state while maintaining its spherical shape then on, as shown in Fig. 3(d). Since the erythrocyte membrane remained in the normal state, the erythrocyte in Fig. 3(d) was within the scope of this calculation.

![Fig. 4 Change in strain energy.](image)

Figure 4 shows the change in strain energy corresponding to the change in volume of the erythrocytes that is depicted by the red solid line in Fig. 3. In Fig. 4, the dotted line depicts the strain energy caused due to bending, while the black solid line depicts the strain energy caused due to shearing, and the red solid line depicts the total strain energy. In the initial process of erythrocyte swelling, the strain energy caused due to bending exceeds that caused due to shearing. However, when the erythrocytes reach a near-spherical shape and exceed $V/V_{max} = 0.88$, the strain energy caused due to shearing increases rapidly and becomes larger than that caused due to bending. This tendency is similar to that of the literature (Pai, et al., 1980). The shape of the erythrocyte at $V/V_{max} = 0.88$ is shown in Fig. 4. Therefore, when modeling
the near-spherical shape in the swelling process of erythrocytes, it is necessary to consider the shear rigidity. This is also shown by the change in erythrocyte pressure in Fig. 5.

Figure 5 shows the change in erythrocyte pressure corresponding to Fig. 4. However, since the pressure increases rapidly when approaching the spherical shape, the vertical axis is displayed as a common logarithm. Further, the dotted line shows the pressure due to bending strain; the black solid line, the pressure due to shear strain; and the red solid line, the sum of the pressures. Here, similar to the trend in Fig. 4, the pressure due to bending exceeds the pressure due to shearing in the initial stage of the erythrocyte swelling process. When the erythrocytes reach a near-spherical shape and exceed \( V/V_{\text{max}} = 0.83 \), the pressure due to shearing exceeds the former. The shape of the erythrocyte at \( V/V_{\text{max}} = 0.83 \) is also shown in Fig. 5. The maximum value of pressure when it becomes spherical is 20.1 Pa. Since the pressure values of normal erythrocytes in spherical shape are reported to be 23 Pa (Rand, et al., 1964), 11.0 Pa (Zarda, et al., 1977), and 29.3 Pa (Pai, et al., 1980), the results of the present calculation are close to those obtained in previous studies.

4. Results and Discussion

Owing to diabetes, the bending and shear rigidity of the erythrocyte membrane increases more than that of normal erythrocytes (McMillan, et al., 1983; Chang, et al., 2017). The shear rigidity of the erythrocyte membrane lesioned by malaria increases 10 times more than that of normal erythrocytes (Zhang, et al., 2015). Further, oxidative stress may increase the bending and shear rigidity of the erythrocyte membrane by approximately five and 100 times, respectively, as a cumulative change occurring in vivo under pathological conditions (Hale, et al., 2011). Therefore, we performed calculations by independently increasing the bending and shear rigidity by 10 times.

Figure 6 shows the effects of increasing the bending rigidity and shear rigidity by 10 times each on the pressure. Figures 3–5 show the results obtained when the bending rigidity is \( D = 1.8 \times 10^{-5} \) Nm and shear rigidity is \( \mu = 6.0 \times 10^4 \) N/m. The red solid lines in Figs. 5 and 6 denote the same pressure, which is shown as \( (D, \mu) \). In Fig. 6, the dotted line shows the result of increasing only \( D \) by 10 times, i.e., \( (10D, \mu) \). The black solid line denotes the result of increasing only \( \mu \) by 10 times, i.e., \( (D, 10\mu) \). Compared with the pressure in \( (D, \mu) \), for \( (10D, \mu) \), the increase in pressure is larger at the initial stage of swelling; and for \( (D, 10\mu) \), the increase in pressure is larger when it is in near-spherical shape and spherical shape.

Figure 7 shows the change in shape of the erythrocytes corresponding to Fig. 6 (only the first quadrant). Compared with the shape in \( (D, \mu) \), the change in shape is small when solely the bending rigidity \( (10D, \mu) \) or solely the shear rigidity \( (D, 10\mu) \) is increased. Pai, et al. (1980) compared the change in shape when the bending rigidity was increased by 10 times that of normal erythrocytes; the change was small. As shown in the calculation result, even when solely the shear rigidity was increased by 10 times, the effect on the shape change was small.

Figure 8 shows the change in the base tension \( T \) of the erythrocytes in spherical shape when the bending rigidity and shear rigidity were increased by 10–100 times. The tension was obtained from the Laplace equation (9) and equation
(16), given below.

![Fig. 7 Comparison of the deformed shapes.](image)

Considering $\alpha$ as the rate of increase of rigidity, the results when only $D$ is multiplied by $\alpha$, and when only $\mu$ is multiplied by $\alpha$ are shown by $(\alpha D, \mu)$ and $(D, \alpha \mu)$, respectively. The point on the left end of Fig. 8 is the result for the reference $\alpha = 1$, that is, $(D, \mu)$. The calculations were performed by considering $\alpha = 10, 20, \ldots, 100$. The dotted lines in Fig. 8 denote the average values of base tension on the surface of the erythrocyte obtained from the force balancing equation expressed by Eq. (16), and the points are connected by lines for easy viewing. Equation (16) is expressed in the Supplement section. The dotted lines correspond to the symbols. In all the two cases, the base tension $T$ increases linearly as $\alpha$ increases.

![Fig. 8 Effect of the changes in bending rigidity and shear rigidity on membrane base tension when the shape is spherical.](image)

When erythrocytes are spherical, the dimensionless quantity $\psi$ in Eqs. (11) and (12) can be zero. Then, Eqs. (11) and (12) can be respectively transformed into

$$
\frac{p_{\text{max}} \ell^3}{D} = \xi \left( \frac{\mu \ell^2}{D} \right)
$$

(14)

$$
\frac{p_{\text{max}} \ell^3}{\mu} = \eta \left( \frac{\mu \ell^2}{D} \right)
$$

(15)

where $\xi$ and $\eta$ are functions obtained by solving Eqs. (11) and (12) with respect to $\pi_1$ and $\pi_3$, respectively, under the condition of $\psi=0$. 

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The magnitude of \( \ell \), the erythrocyte diameter, is constant in the spherical state independent of \( D \) and \( \mu \), because the surface area remains unchanged during deformation from the initial state. When \( D \) is constant, \( p_{\text{max}} \) is a function of only \( \mu \) from Eq. (14), and when \( \mu \) is constant, \( p_{\text{max}} \) is a function of only \( D \) from Eq. (15). On the other hand, when erythrocytes having different \( D \) and \( \mu \) values swell from an initial shape to the same sphere, the changes in both principal curvatures and shear strains are the same for these erythrocytes independent of the values of \( D \) and \( \mu \). This observation is derived from the linear strain energy model of Eqs. (3) and (4) with respect to \( D \) and \( \mu \), respectively, and confirmed from the calculated results of the bending and shear strains at the spherical state. Therefore, when \( D \) is constant, \( p_{\text{max}} \) is a function of only \( \mu \), and when \( \mu \) is constant, \( p_{\text{max}} \) is a linear function of \( D \). These results and Eq. (9) give the linear relationship between \( T \) and \( \alpha \), as depicted by the solid and open circles in Fig. 8, respectively.

In Fig. 8, the value of the leftmost minimum tension corresponding to \( \alpha = 1 \), is equal to 0.034 mN/m. When \( \alpha \) is increased to 100, the value of the rightmost maximum tension for the solid circle is slightly less than 100 times the value of the minimum tension. Therefore, the base tension caused by shear increases linearly with increase in the shear rigidity \( \mu \), but this increase ratio is slightly less than the increase ratio of shear rigidity.

In Zarda, et al., (1977), the value of \( \pi_2 \) was 50 for the normal erythrocyte, in which the reference length was chosen as \( \ell = 1 \mu \text{m} \). If this value of \( \ell \) is used in our calculation, \( \pi_2 \) becomes 33.3 for the normal erythrocyte.

Initially, the erythrocytes swell and become spherical with a constant surface area; then, as the swelling progresses, the surface area increases, and hemolysis occurs when the tension reaches the specified limit. The tension level at which hemolysis occurs is 15 mN/m (Rand, 1964; Canham, et al., 1970), which can be used for normal erythrocytes, neglecting the base tension at which the erythrocyte first becomes spherical. However, for where the bending and shear rigidity are increased due to lesions or oxidative stress, to determine the tension level at which hemolysis will occur, the base tension at which the erythrocytes become spherical should be considered. Figure 8 shows that the effect of bending rigidity on the tension level that causes hemolysis is negligible even when its value is increased to 100 times that of normal erythrocytes. On the contrary, if the shear rigidity is increased to 50–100 times that of normal erythrocytes, the base tension becomes approximately 1.5 and 3.0 mN/m, respectively. Then, the tension level at which hemolysis occurs needs to be reduced by 10% and 20%, respectively.

5. Conclusion

We evaluated the swelling process of erythrocytes when applying osmotic shock to the erythrocytes using a hypotonic solution. To this end, the total strain energy due to bending strain and shear strain was minimized. The bending and shear rigidity of the erythrocyte membrane increases owing to lesions and oxidative stress. We calculated the increases in internal pressure and membrane tension due to the increase in bending and shear rigidity, and then re-evaluated the tension level at which hemolysis occurs by considering the base tension for spherical erythrocytes. In conclusion, we obtained the following results:

(1) When solely the bending rigidity or solely the shear rigidity is increased, the base tension of the membrane when erythrocyte is spherical increases linearly. The base tension caused by only shear increases linearly with increase in the shear rigidity; however the increase ratio of base tension is slightly less than that of shear rigidity.

(2) In normal erythrocytes, the tension at which hemolysis occurs is 15 mN/m. Even if the bending rigidity is increased to 100 times that of normal erythrocytes, the effect on the tension at which hemolysis occurs is negligible. However, when the shear rigidity is increased to 50–100 times that of normal erythrocytes, it is necessary to reduce the hemolysis tension level by 10% and 20%, respectively.

Supplement

The base tension depicted by the dotted lines in Fig. 8 denotes the following Eq. (16). The isotropic tension \( T \) can be obtained from the following equilibrium equation of forces in the normal direction of the membrane (Timoshenko, et al., 1959):

\[
T = \frac{1}{\sqrt{2}} \left[ \frac{p_{\text{max}} - p_{\text{min}}}{2} \right] \left( \frac{1}{\sqrt{1 + \alpha^2}} \right)
\]
\[ T = \frac{1}{\kappa_s + \kappa_p} \left[ p - \kappa_s T_s - \kappa_p T_p - \frac{d(rq)}{rds} \right] \]  

(16)

where \( T_s, T_p \) are the meridional tension and circumferential tension, respectively, and \( q \) is the out-of-plane shear force. Each of these can be represented as follows:

\[ T_s = \frac{\mu}{2} \left( \lambda^2_s - \lambda_s^2 \right), \quad T_p = -T_s \]  

(17)

\[ q = m_s - m_v \frac{dr}{ds} + \frac{dm_v}{ds} \]  

(18)

where \( m_s, m_v \) are the moments corresponding to the principal curvatures \( \kappa_s, \kappa_v \), respectively, and can be obtained as follows:

\[ m_s = D(\kappa_s - \kappa_{0s}), \quad m_v = D(\kappa_v - \kappa_{0v}) \]  

(19)

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