Generation Mechanism of Vascular Endothelial Chained Vesicles and Transendothelial Channel*

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Vascular endothelial cell vesicles are attached to both luminal and abluminal surfaces of the endothelium and contribute to the transport of specific macromolecules between the external environment and the cell. The vesicles have mostly flask-like or chained bead-like shapes, while they appear free within the cytoplasm. The existence of a transendothelial channel, which is considered to be involved in specific transport systems, is also reported. In this study, the generation mechanism of vascular endothelial chained vesicles and the transendothelial channel is theoretically investigated based on a method proposed by J.C. Luke (1982). A system of nonlinear differential equations is derived according to the variational principle and is reduced to a two-point boundary value problem. The equations are solved using the shooting method along with the Newton-Raphson and Runge-Kutta methods. The computed shape of the vesicle suggests that the chained vesicles and the cylindrical transendothelial channel are the most dynamically stable shape which can be formed in vascular endothelial cells.

Key Words: Vascular Endothelium, Chained Vesicles, Transendothelial Channel, Generation Mechanism, Shape Analysis, Membrane Shell

1. Introduction

As schematically shown in Fig. 1, the inner wall of a normal blood vessel is covered by a monolayer of numerous endothelial cells (by three cells in the Fig. 1). Some capillaries are comprised of a single endothelial cell. It is the only interface between blood flow and tissues, and highly active metabolic process occur in the endothelial cell. Vascular endothelial cell vesicles, most of which are between 50 to 100 nm in

diameter, are attached to both luminal and abluminal surfaces of the endothelium and are intimately involved with microcirculation⁽¹⁾⁻⁽³⁾ of cells and tissues. Selective transport of macromolecules such as

Fig. 1 Schematic drawing of vascular endothelial cell and vesicles

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ingestion of proteins and bacteria, as well as secretion of enzymes and hormones, is not achieved by passage across the plasma membrane but by the so-called exocytosis and endocytosis⁽³⁾⁻⁽⁵⁾ which are accompanied by a large deformation of the membrane vesicles, as shown in Fig. 2. Furthermore, there are several experimental observations^{(6),(7)} which give evidence for the presence of the chained vesicles and the transendothelial channel (we call this the "channel" hereafter). These are considered to contribute to the specific transport of macromolecules. Few details, however, are known about their mechanics.

If these functions were blocked, cell death could be triggered and then the tissues of blood vessel walls could be damaged. This causes abnormal microcirculation which, in turn, leads to various diseases⁽⁸⁾ such as atherosclerosis⁽⁹⁾. The risks for such fatal diseases have been increasing not only in western countries but also in Japan recently. It is predicted that this situation could be developed more significantly as the average life span increases. Thus, the elucidation of the basic mechanisms of these diseases is of fundamental importance in medicine.

So far, a large number of studies⁽¹⁰⁾⁻⁽¹²⁾ have been carried out on the deformation and shape analyses of completely closed vesicles such as red blood cells. However, few details are known about the mechanical characteristics of endothelial cell vesicles. In this study, therefore, the generation mechanism of the vascular endothelial chained vesicles and the transendothelial channel is theoretically investigated following our previous studies^{(13),(14)} of single vesicles.

Fig. 2 Schematic drawing of probable generation sequence of free vesicle (*Fv*), chained vesicles (*Cv*) and transendothelial channel (*Tc*)

2. Theoretical Development

2.1 Fundamental assumptions

The endothelial cell is considered to have spontaneous curvature, which will be discussed later. Therefore, from a mechanical viewpoint, the vesicle or the chained vesicles due to the spontaneous curvature can be easily formed in an unstressed or in almost unstressed conditions. These vesicles not only contribute to vesicular transport but also serve as a membrane reservoir for controlling excess membrane⁽¹³⁾. The channel is considered to be formed by the fusion of vesicles or chained vesicles grown from the luminal side or the abluminal side, or from both sides of the endothelial cell. The mechanical factors that initiate and control these phenomena are the state of cortical stress, or cortical tension, the amount of excess membrane, the spontaneous curvature and the transmural pressure between the inside and the outside of the membrane.

Most vesicles formed in cells other than endothelial cells are coated vesicles which are covered by clathrin, a basket-like protein coat structure. Endothelial cell vesicles formed in rather narrow blood vessels, however, do not have such coat structure and are composed of only the plasma membrane⁽³⁾. In this study, the endothelial cell vesicle is assumed to be a homogenous isotropic membrane shell. Also, the vesicle membrane is assumed to be a typical lipid bilayer membrane to show two-dimensional fluid characteristics, and the surface area of the membrane is assumed to be constant⁽¹¹⁾⁻⁽¹³⁾ during deformation. These assumptions are often used in the analysis of red blood cell membranes. That is, various methods such as the micropipet aspiration method and theoretical analysis have revealed that the red blood cell membrane is a lipid bilayer membrane and can be mechanically modeled as a thin elastic membrane surrounding a viscous liquid which deforms at a constant surface area⁽¹⁵⁾. There exists the effect of spectrin which underlies the red blood cell membrane as the structural meshwork. Therefore, it is somewhat controversial that these assumptions are directly applied to the endothelial cell membrane vesicle⁽¹⁶⁾. These are utilized, however, in this study as a first step. Also, the deformation is assumed to be sufficiently slow so that shear stress is negligible.

If the vesicle is assumed to be an elastic shell, the so-called three-dimensional finite deformation theory should be used, although the analysis will be extremely complicated. On the other hand, the method proposed by Luke⁽¹⁷⁾ for shape analysis of completely closed vesicles such as red blood cells is highly important as it can provide a simple way to study the large

deformation shape analysis for membrane structures. In this study, the fundamentals of the basic equations in Luke's method are introduced so as to apply the method to vesicles with openings.

2.2 Fundamental equations

The coordinate system x - z , the angle θ , the radius r , and the distance s are defined on a segment Q_0Q of a cross section of an axisymmetric membrane shell shown in Fig. 3. T is the radial tension at end perimeter Q in the vertical direction; p , the pressure difference between external and internal compartments; A , the surface area; V , the volume; D , the bending stiffness; c_1 , c_2 , the principal curvatures of the membrane surface and c_0 , the spontaneous curvature in the resting state. General boundary conditions at both ends of the membrane are:

$$\begin{aligned}\theta(0) &= \theta_i, \quad r(0) = r_i \\ \theta(A_t) &= \theta_t, \quad r(A_t) = r_t\end{aligned}\quad (1)$$

where θ_i , r_i and θ_t , r_t are the constants to be determined according to the starting and ending boundary conditions, respectively. Subscripts i and t stand for starting (initial) and ending (terminal) boundary states on the vesicle cross section. These are used in the following equations. The functional for the membrane vesicle is defined as follows:

$$\begin{aligned}E_B &= \int_0^{A_t} \frac{D}{2} (c_1 + c_2 - c_0)^2 dA \\ &+ p \left(\int_0^{A_t} \frac{1}{2} r \cos \theta dA - V_t \right) \\ &+ T_t \left(\int_0^{A_t} \sin \theta dA - \pi r_t^2 \right)\end{aligned}\quad (2)$$

where the first term is the bending strain energy (we call this the bending energy), and the second and third terms are the constraints with respect to volume and radius, respectively. Also, p and T_t serve as Lagrange multipliers. The constraint of constant membrane surface area is automatically satisfied by using A as

Fig. 3 Coordinate system taken on a vesicle cross section

an independent variable. The condition is set to seek the equilibrium shape of the vesicle which makes the bending energy minimum while satisfying these constraints. That is, the variational principle $\delta E_B = 0$, the minimum condition of the functional, yields the following Euler equations.

$$\begin{aligned}\frac{dB}{ds} &= \frac{1}{D} \left(\frac{1}{2} p r \sin \theta - T \cos \theta \right) \\ \frac{dT}{ds} &= D \left(\frac{2B}{r^2} \cos \theta - \frac{B^2}{r} - \frac{B c_0}{r} \right) - \frac{1}{2} p \cos \theta\end{aligned}\quad (3)$$

where,

$$\begin{aligned}T(A) &= T_t + \int_A^{A_t} \frac{1}{(2\pi r)} \\ &\times \left(\frac{DB^2}{r} + \frac{DBc_0}{r} - 2 \frac{DB}{r^2} \cos \theta + \frac{1}{2} p \cos \theta \right) da\end{aligned}\quad (4)$$

$$B = c_1 + c_2 - c_0 \quad (5)$$

The geometrical variables in Fig. 3 are related by the following Eqs. (6) and (7):

$$\frac{d\theta}{ds} = \frac{\cos \theta}{r} - c_0 - B, \quad \frac{dr}{ds} = \sin \theta \quad (6)$$

$$\frac{dz}{ds} = \cos \theta, \quad \frac{dA}{ds} = 2\pi r$$

$$\frac{dV}{ds} = \pi r^2 \cos \theta, \quad \frac{dE_B}{ds} = D\pi r B^2 \quad (7)$$

Equations (3) and (6) are solved simultaneously. Then, Eqs. (7) are used to compute vesicle shapes, surface area, volume and energy.

3. Numerical Computations

The simultaneous nonlinear differential equations in (3) are solved by numerical integration since these cannot be solved analytically. The fourth-order Runge-Kutta method was used. The initial values of θ and r are geometrically determined from the boundary conditions (1) for the case of the vesicle with openings. On the other hand, T_i and B_i , the initial values of T and B , cannot be determined directly from the boundary conditions. These can be iteratively specified so that the terminal boundary conditions are satisfied. This type of problem is called a two-point boundary value problem and the shooting method⁽¹⁸⁾ is used here to solve the problem numerically.

In the method, the desired initial values are set randomly. The discrepancies from the desired boundary condition at the other end point are used to adjust the initial values until the boundary conditions at both end points are ultimately satisfied. In this study, we first select randomly the assumed values of T_i and B_i , and then, Eqs. (3) and (6) are integrated with the aid of the Runge-Kutta technique to determine the values of that terminal boundary condition. The discrepancies between the calculated boundary conditions

and the desired boundary conditions are defined as F , which is now considered as the function of T_i and B_i . Then, the Newton-Raphson method is used in order to seek T_i and B_i that ultimately satisfy the equation $F(T_i, B_i)=0$.

By using computed values of T_i and B_i , Eqs. (3) and (6) are solved again using the Runge-Kutta technique to yield the deformed shapes and their parameters at the end points. The computations presented here were carried out on OMRON AV Series WORK STATION with double precision arithmetics.

4. Numerical Examples

Based on observed results⁽³⁾⁻⁽⁷⁾, Fig. 2 shows a schematic drawing of the probable formation mechanisms of a free single vesicle (Fv) or chained vesicles (Cv) in which the plasma membrane of the endothelial cell invaginates and ingests macromolecules, and then these settle into the transendothelial channel (Tc). In the selective ingestion of macromolecules by chemical recognition as represented by the immune system, for example, active changes are initiated in some of the mechanical factors such as cortical tension, the amount of excess membrane, the spontaneous curvature and the transmural pressure by the preceding production of some chemical recognition signals. As a result of active control by these factors, vesicular transport of the selective ingestion is generated by a sequential process of invagination of the plasma membrane, forming a pit and then a vesicle, and finally pinching off from the membrane.

A model of repeated units of attached vesicles is assumed in Fig. 4 in order to develop a theoretical analysis for the formation of the chained vesicles and the channel shown in Fig. 2. In this study, as a first step, the generation mechanism of one repeating unit and one bottom unit in the chained vesicles shown in Fig. 4 is investigated separately. That is, stable shapes with respect to energy will be presented for the repeating units which are continuously connected to adjacent units at the boundaries. The channel is assumed to be formed by connected repeating units. The independent variable s of Eqs. (3) and (6) was transferred into surface area A with the use of Eq. (7) because of computational simplicity.

Experimental data of membrane bending stiffness of endothelial cell vesicles are so far not available. Therefore, in this study, the bending stiffness is assumed to be equal to that of a similar bilayer membrane of red blood cell^{(11),(13)}, i.e., $D=10^{-12}$ dyn cm. The pressure difference and the initial radius are assumed to be as $p=1\,000$ dyn/cm² ($=1.0$ cmH₂O)⁽¹³⁾ and 66.7 nm, respectively.

Fig. 4 Assumed model of chained vesicles provided for analysis

4.1 Repeating units

From the repeating units shown in Fig. 4, the geometric boundary conditions with respect to r and θ , and the initial condition of B are set as follows:

$$\begin{aligned}\theta(0)=0, \quad r(0)=r_i \\ \theta(A_0)=0, \quad B(0)=0\end{aligned}\quad (8)$$

where A_0 is the surface area of the initial spherical vesicle. Since the value of θ at both lower and upper ends be zero from Eq. (8), the equilibrium shapes were computed with the value of r_i was changed in a step-by-step manner.

The computed unfolding sequence of a repeating unit is shown in Fig. 5. The figure shows the right side of each vesicle cross section. Shape 1 shows the initial spherical vesicle, while membrane contours 2, 3, ..., 15, 16 correspond to the shapes of the vesicle with increasing opening radius. Number 7 is almost a cylindrical tube, the length of which is about $4r_0$ and the radius at the lower end is about $0.5r_0$. Once the opening radius becomes slightly larger than that of number 7, at number 8, suddenly there appears a tight constriction on the middle part in the axial direction of the cylindrical tube. The cause of this buckling-like phenomena might be the presence of a spontaneous curvature. When the value of r_i is increased further to around r_0 (number 12), the vesicle settles into a so-called hour-glass shape which has a tight constriction on the middle part and is symmetric in the vertical direction.

Figure 6 shows the relationships of the nondimensional lower opening radius with bending energy, volume, surface area, and in-plane tensions at both perimeters. The surface area remains constant as assumed, while slight changes are observed in the

volume. It is interesting to observe that the bending energy takes a minimum at numbers 7 and 12, which correspond to opening radii of $0.5r_0$ and r_0 , respectively. This indicates that these two shapes and their adjacent ones are, similar to the initial spherical vesicle, stable with respect to energy among the probable shapes of the vesicle with spontaneous curva-

Fig. 5 Unfolding sequence of a repeating unit

Fig. 6 Relationships of lower opening radius with bending energy, in-plane tensions, surface area and volume of the repeating unit ($r_0=66.67$ nm)

ture c_0 . Therefore, once excess membrane, due to such factors as local activation of metabolism, is provided to the cytoplasmic vesicles with spontaneous curvature c_0 , chained vesicles or a cylindrical channel derived from the chained vesicles may be formed. Some experimental observations^{(6),(7)} have given evidence for the presence of the transendothelial channel which is somewhat distorted and its shape is something in between an hourglass and a cylindrical tube.

Figure 7 shows three-dimensional representations of the unfolding sequence of a spherical vesicle. They are drawn using Mathematica, incorporating computed data given in Fig. 5. These images are shown upside down with respect to their counterparts in Fig. 5, so that the opening radius can easily be observed; the numbers correspond to those in Fig. 5. Smooth yet dramatic shape changes from spherical vesicle to cylindrical tube and then to hourglass are clearly observed. Buckling like phenomena due to the effect of the spontaneous curvature are particularly observed from numbers 1 to 2, 7 to 8 and 13 to 14. Numbers 1, 4, 5, 6, 7, 11 and 12, the shapes with comparatively lower bending energies, are considered as one part of the potentially actual shapes of the repeating unit shown in Fig. 4 during the transient process from a single vesicle to chained vesicles and then to the cylindrical channel.

4.2 Bottom unit

Next, the shapes of the bottom unit of the chained vesicles are investigated. The following boundary conditions are set by considering the opening end to be smoothly connected to the lower end of the repeating unit and the other end to be closed, as shown in Fig. 4.

$$r(0)=r_i, \quad \theta(0)=0$$

$$r(A_t)=0, \quad \theta(A_t)=-\frac{\pi}{2} \quad (9)$$

The value of the opening radius r_i was restricted near inside of r_0 . Solutions are sought with the surface area changed in a step-by-step manner around $1.5A_0$ because of computational simplicity.

Figure 8 shows the computed relationships between the bending energy and the surface area. With surface area taken as the variable, curves for each opening radius exhibit a minimum energy point. Each minimum energy point was determined to be the solution of that opening radius. From the figure, the most stable shape with respect to energy for the case of the opening radius $1.00r_0$ is the membrane having spontaneous curvature c_0 and surface area $1.5A_0$.

Figure 9 shows the typical computed shapes. Each shape represents the minimum bending energy equilibrium configuration for a given opening radius. When the opening radius approaches r_0 , the distance between the opening and the bottom decreases. This

Fig. 7 Three-dimensional graphic expressions of the unfolding sequence of a repeating unit. These graphic images were drawn using Mathematica, incorporating computed data given in Fig. 5

Fig. 8 Relationships between bending energy and surface area of the bottom unit

distance becomes $3r_0$ when the opening radius completely coincides with r_0 , and the constriction becomes extremely deep. Although not clearly seen in Fig. 9, there exists a pin-like hole at the constricted part.

Figure 10 shows the relationships of the minimum bending energy and the in-plane tension T_t with the

Fig. 9 Equilibrium shapes of the bottom unit with different opening radii

opening radius. Bending energy E decreases as the opening radius approaches r_0 and is almost zero when the opening radius completely coincides with r_0 . The value of tension T_t is minus, which means the force is acting inwardly, and the absolute value of T_t decreases as the opening radius increases. This tendency is the same as that of the repeating unit around

Fig. 10 Relationships of bending energy and in-plane tension with opening radius of the bottom unit

the opening radius is r_0 . Tension T_t becomes zero when the opening radius completely coincides with r_0 . This shape, i.e., number 3 in Fig. 9, is, therefore, the most stable shape with respect to energy and is the desired solution of the bottom unit.

5. Discussion

A method of analysis based on membrane bending strain energy has often been utilized to investigate the mechanics of red blood cell membrane because of its simplicity. Luke's method lies in the same category and is applied in this study. A method to analyze the mechanics of the chained vesicles and the channel was developed, where modeling was focused on the specific configuration of the chained vesicles with repeating units. It is expected that the mechanics of the vesicles and the endothelial cell membrane are thoroughly investigated by the modification and systemization of the present, comparatively simple method. As regards the membrane bending stiffness, on the other hand, it is assumed to be equal to that of the red blood cell membrane, although the validity of this is still somewhat controversial. The results newly obtained in this study and the related discussions can be summarized as follows.

(1) When the opening perimeter of a spherical membrane shell is unfolded with its perimeter kept parallel to its axis, there exists several points where the shapes and the tensions change significantly with slight changes of the opening radius. This is considered to be the buckling-like phenomena of the mem-

brane with a spontaneous curvature.

(2) In the repeating units of chained vesicles, it is found that the cylindrical tube shape and the hourglass shape are stable with respect to energy.

(3) In the bottom unit of the chained vesicles, it is found that the hourglass shape with a constriction is stable with respect to energy.

Consequently, it is theoretically suggested that the chained vesicles and the cylindrical channel derived from the chained vesicles are some of the stable shapes with respect to energy and can potentially be formed and presented in the vascular endothelial cell.

A lot of problems remain unsolved, such as how to identify the stiffness of the endothelial cell membrane and to estimate of its effect, how to measure the value of cortical tension, and how to estimate membrane shear stress and its effect upon whole-cell deformation. In the case of complicated problems, the method presented in this study is limited and the finite element method developed for the mechanics of cellular membrane is needed. Since the generation of vesicles and channels is considered to be dynamic biological activities of the nanometer order, the direct in vivo observations and analysis based on these results might ultimately be required. In order to realize these investigations, the development of new electron microscope facilities as well as nano manipulator systems is highly desired.

6. Conclusion

In this study, the generation mechanisms of vascular endothelial chained vesicles and transendothelial channel have been theoretically investigated based on the elastic strain energy of the membrane. As a result, dramatic changes of shapes and cortical tensions of the vesicle were found and some buckling-like phenomena of the membrane due to spontaneous curvature were also observed. It is theoretically suggested that the hourglass-shaped chained vesicle and the cylindrical tube-shaped channel are some of the dynamically stable shapes with respect to energy and can potentially be formed in the vascular endothelial cell.

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