Mechanical Properties of Breast Cancer Cell Membrane Studied with Optical Tweezers *

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Membrane tethers are extracted from breast cancer cells using a force generated by an optical trap. It is experimentally obtained that the radius of tether is about 0.1 μm and the static tether force is about 8.5 pN. Calculations based on the experimental measurements give a bending modulus for the tether of 1.35×10^{-19} N·m and a surface membrane tension of 6.76×10^{-6} N/m in the breast cancer cell. The treatment with cytochalasin D results in the decreasing bending modulus and decreasing apparent surface tension. When the membrane protein caveolin is over-expressed, similar cases occur in bending modulus and apparent surface tension. In addition, the viscous resistance coefficient of the membrane is calculated to be 1.15 pN·s/μm according to the dynamic tether forces obtained under different pulling velocities.

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The membrane is a material barrier that isolates the cell from the exterior environment. It also functions as a modulator of cell membrane permeation. Almost all the activities of the cell are related to the cell membrane. The mechanical structure of biological membrane is a very important property distinguished from the chemical one. It reflects the characteristic of membrane from the view of its component and assembly. Much attention has focused on how a cell regulates its migration, deformation, exocytosis, endocytosis and response to the shear force exerted by blood flow and how the change of the cell membrane components affects the mechanical property.

Several experimental methods have been used to investigate the mechanical properties of cell membranes.[1−5] Using these methods, the mechanical properties of liposomes, sea urchin eggs, erythrocytes, and lymphocytes have been studied. However, they are applicable mainly to suspension cells and are inapplicable to cells with complex cell structures. Optical tweezers allow exquisitely fine control of a wide size range of particles in a noninvasive manner. They have been used for a variety of applications including the measurement of membrane barriers, the force of single motor molecule and regional specializations in cell membranes.[6−8] In this Letter, the mechanical properties of the breast cancer cell membrane have been studied and several parameters such as membrane surface tension, viscous resistance coefficient and bending modulus are obtained.

The optical tweezer system consists of an inverted optical microscope (Leica DMIRB, Germany) with a high numerical aperture objective (HCXAPO 100× NA 1.30), Ti–sapphire laser (3900S, American Spectra Physics Inc.) and three-dimensional piezo-electric driven stage (NIS-70, Israel) with a maximal tunable range of 70 μm and sub-nanometre resolution. The image of sample is projected onto the surface of both the CCD (charge coupled device) camera (CoolSNAP-fx, America) and the quadrant photodiode detector (Hamamtsu 1557-03). The schematic diagram of the system can be seen in Ref. [9] and the detailed description of the quadrant detector can be seen in Ref. [10]. To prepare lectin-coated polystyrene beads, 50 μL suspension of beads with concentration of 5% is sonicated for 10 min then mixed with 10 μL (0.1 mg/mL) lectin. The mixture is incubated for two hours at room temperature and oscillated during this process. Finally the beads are washed three times with PBS and resuspended in 200 μL PBS buffer.

Breast cancer cells Hs578 are grown in a 37°C CO_2 incubator with 5% CO_2 and saturated humidity. Culture medium is made up of 10% bovine serum, 2 g/L $NaHCO_3$, 100 u/mL penicillin and RPMI-1640 of 100 u/mL streptomycin. Synchronization of cells must be carried out before experiments. After generation, cells are incubated overnight then displaced to a 4°C refrigerator for 8 h and then incubated for 16–17 h in a 37°C incubator.

The suspended cells cannot be deformed with a single laser trap, so they must be attached to the sample cell. In our experiments, the breast cancer cells

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are attached to the bottom of sample cell with poly-l-lysine. The adhesion of bead and breast cancer cell is performed as follows. First, a bead is trapped and we move the stage to make a cancer cell close to the bead. Then we adjust the focal plane of objective to ensure that the bead and breast cancer cell are parfocal. When a stable combination is formed, we drag the cell away from the bead. When the bead is displaced from the cell membrane, a tether is formed between the bead and membrane. Its diameter is about 0.2 μm. The force exerted on it can be obtained by measuring the displacement of the bead from the trap centre. In our system, a quadrant photodiode detector is used for tracking the bead movement and a high-resolution cooled CCD camera is for monitoring and acquiring the whole image of the bead and cell.

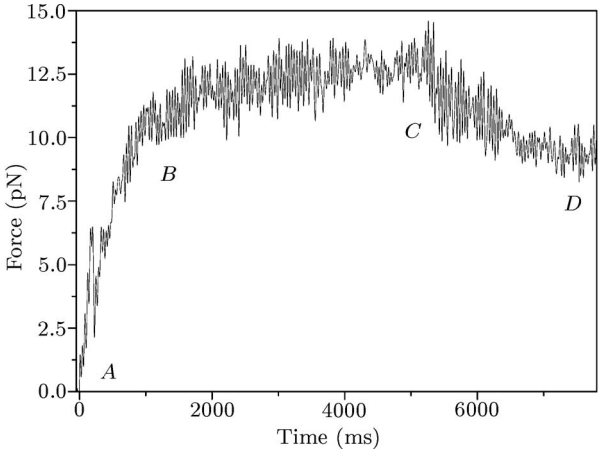

Fig. 1. Force of the membrane tether vs time during tether elongation.

The curve of the force vs time during tether elongation is shown in Fig. 1. It can be divided into three parts: initial part (A–B), elongation part (B–C) and static part (C–D). Before the point C the scanner moves with a constant velocity of 2.2 μm/s, so these two parts reflect the dynamic process of the tether formation. After the point C the scanner stops moving, accordingly this part represents the transition from a dynamic state to the static one.

During the initial part, the tether force increases sharply. This shows that the lipid membrane is hard to be extended. When an external force is exerted to deform the cell membrane, the membrane tension will increase to resist the change. After the point B, the tether force increases slowly with the further elongation of tether, suggesting that the membrane is drawn from a reservoir. A membrane reservoir is usually related to membrane folds and invaginations. It acts as a buffer that prevents the membrane tension from changing, which is significant to the cell function and activity. The function of tether force vs time can be written as[11]

$$F = a + b\ln\left(1 + \frac{t}{c}\right), \quad c = \frac{\rho R_t}{2V}. \tag{1}$$

Then the slope is

$$k(t) = \dot{F}(t) = \frac{b}{c+t} \tag{2}$$

and

$$|\dot{k}(t)| = \frac{b}{(c+t)^2}. \tag{3}$$

When $t = 0$,

$$|\dot{k}(0)| = \frac{b}{c^2}$$

and when $t > t' = 3c$,

$$|\dot{k}(t)| < |\dot{k}(t')| = \frac{b}{c^2} \times 6.25\%,$$

the part of $t > t'$ can be considered to be linear, and the point $F(t')$ is defined as point B. Point B seems to be a characteristic point, which indicates that the reservoir takes effect. After that, the membrane tension will remain almost constant. The increase of tether force should derive from the resistance of friction between the motional membrane and its surrounding.

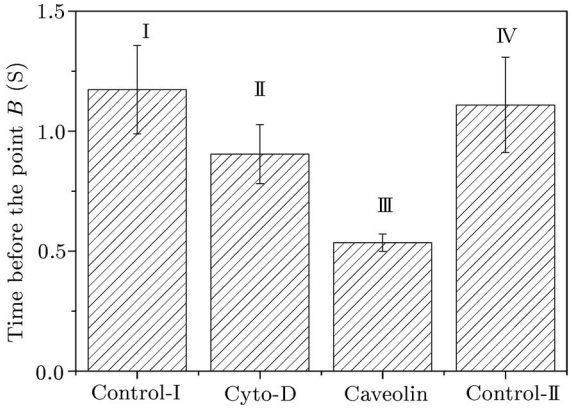

Fig. 2. Expended time during the initial part before and after treatments with cytochalasin D, caveolin being over-expressed and with different velocities.

Figure 2 shows the expended time during the initial part ($A \sim B$) of the tether formation under different conditions. I corresponds to the control condition and the tether is pulled at the velocity of 2.2 μm/s. II is under the condition that the cell is treated with cytochalasin D (10 μM) and III is that the caveolin is over-expressed on the cell membrane. The pulling velocities both in II and III are 2.2 μm/s. IV is also under the control condition, but the difference is that the pulling velocity in IV is 1.1 μm/s. It can be seen that the lapsed time decreases by 23% after treatment with cytochalasin D and 54% when membrane protein caveolin is over-expressed. In addition, when the pulling

velocity decreases by one-half, the time remains almost constant. Because this initial part reflects the response speed of membrane reservoir, the results indicate that on the one hand, the response time of the membrane reservoir under control condition is almost unchanged, and on the other hand, the response time decreases when the cell is treated with cytochalasin D which can inhibit actin polymerization or the cell membrane protein caveolin is over-expressed.

Figure 3 shows the dynamic tether forces under the four different conditions mentioned above. Because the dynamic force in the elongation part is not a constant, we take the force just after point B as a standard to compare. It can be seen that the tether dynamic force decreases by 35% after treatment with cytochalasin D and 29% when caveolin is over-expressed. Furthermore, the viscous resistance coefficient of membrane b, which is defined as $b = F/v$, can be obtained according to the dependence of the dynamic force on the pulling velocity, where F is dynamic tether force, and v is the pulling velocity during tether formation. In our experiment, the calculated viscous resistance coefficient of breast cancer cell membrane is $1.15 \pm 0.13\,\mathrm{pN{\cdot}s/\mu m}$.

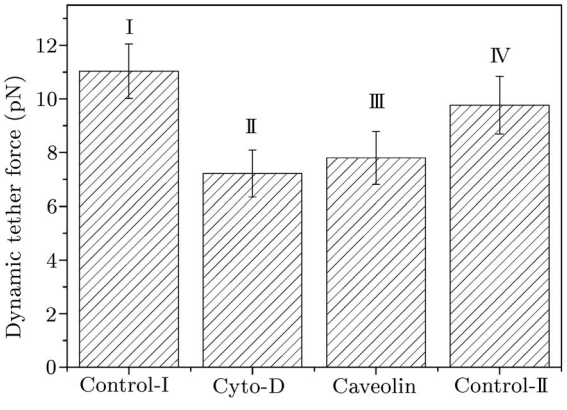

Fig. 3. Dynamic tether forces after the initial phase under the conditions as the same as in Fig. 2.

Sheetz *et al.*[12−14] carried out some studies on tether formation from neuronal growth cone and fibroblast membranes. They put forward a model to deduce the relations among the static tether force, membrane tension and bending modulus. In addition, the concept of qualitatively measuring membrane reservoir by the maximal tether length is also proposed.[14] However, the force profile in the elongation phase in their experiment is different from ours. In their experiment, the force is constant in the elongation period while ours is not constant but a little ascending. According to our experimental results, Jiang *et al.*[11] gave a new mathematical model which is suitable for analysing the tether formation from the cell membrane with reservoir.

Figure 4 shows the static tether force which corresponds to the end of the static part. Similarly, the static force also decreases after treatment. Based on the static force and the radius of the tether, the bending modulus and the membrane tension can be calculated by[15]

$$B = F_0 R_t / 2\pi, \tag{4}$$

$$T_m = F_0 / 4\pi R_t, \tag{5}$$

where F_0 is the static tether force, R_t is the radius of the tether, and T_m is the membrane tension. According to the experimental results, the static tether force of the breast cell membrane under the control condition is $8.5 \pm 1.2\,\mathrm{pN}$, the radius of the tether is about $0.1 \pm 0.02\,\mu\mathrm{m}$, which is measured by magnifying and imaging the tether on the CCD camera. Its precision is determined by the magnification of the image. In our system, the magnification of image is 500. The diameter of the tether is obtained by measuring the number of pixels of the tether image. The resolution of the tether image is one pixel that corresponds to 13.4 nm in real scale. The standard deviation of the tether radius is calculated from the average of different tethers. Therefore, the bending modulus and the membrane tension are calculated to be $(1.35 \pm 0.46) \times 10^{-19}\,\mathrm{N{\cdot}m}$ and $(6.76 \pm 2.30) \times 10^{-6}\,\mathrm{N/m}$, respectively.

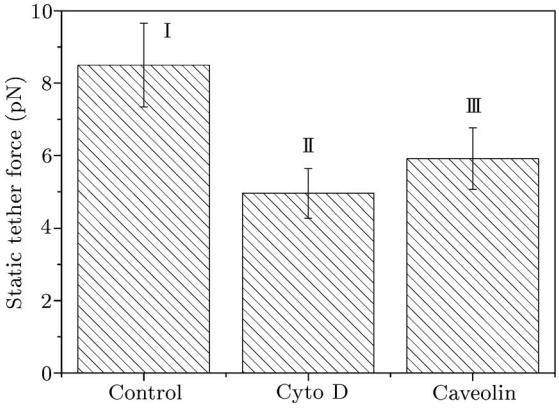

Fig. 4. Static tether force before and after the treatments with cytochalasin D and caveolin being over-expressed.

The breast cell is a type of secreting cell and the membrane reservoir could buffer variations in membrane tension when the secreting cells are stimulated. In our experiments, by studying the process of tether formation, some basic parameters of breast cancer cell membrane are obtained. Moreover, the response time of the reservoir becomes shorter after treatment with cytochalasin D. The function of cytochalasin D is to inhibit actin polymerization. Because the cytoskeleton plays an important role in limiting the size of the available membrane reservoir, the treatment will result in the increase of the available reservoir. Similarly, both the dynamic and static tether forces get smaller compared with untreated one. When the

membrane protein caveolin is over-expressed, the results similar to that after treatment with cytochalasin are obtained. Over-expression of caveolin will cause the increase of cave in cell membrane. In other words, this will increase the membrane reservoir. Consequently, the response time and the tether force get decreased. Our experimental results are in good agreement with the theoretical prediction.

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