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Measuring Piconewton Forces and Its Application in Cellular and Molecular Biomechanics

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Abstract: In the last two decades, the biophysical study of single molecules and cells has been revolutionized by our technical ability of measuring or imposing forces at a piconewton level. In this paper, we present a brief overview of all the techniques that have been developed since the nineteen eighties and some of their applications in cellular and molecular biomechanics. The micropipette aspiration technique and its application in human neutrophil rolling on the endothelium are discussed in more detail.

Protein deformation, protein folding, and protein interactions with other proteins or substrates are all mediated by four fundamental types of minute forces including van der Waals forces, electrostatic forces, hydrogen bonding forces, and hydrophobic interactions. Thus, the forces that are involved in rupturing a single receptor-ligand bond, extending a single molecule like deoxyribonucleic acid (DNA) or titin, or deforming a subcellular structure like a neutrophil microvillus are all at a piconewton level (1-7). Measuring or imposing these forces are crucial for our understanding of molecular and cellular functioning mechanisms. Therefore, various techniques have been developed and successfully applied to a myriad of biomedical problems. These techniques include the microneedle technique, the atomic force microscope (AFM), the optical tweezers, the magnetic force apparatus, the biomembrane force probe (BFP), the flow chamber assay, and the micropipette aspiration technique (MAT).

1. The Microneedle Technique

The microneedle technique, which is based upon the cantilever beam theory in solid mechanics, was first employed in studying the sliding force between two microtubules (8). The stiffness of the microneedle, which can be as small as 0.09 pN/nm, is calibrated by monitoring the thermal motion of its tip (9), by pushing against another microneedle that has a known elastic constant (8), or by being pulled with a known force from an inflated erythrocyte in a micropipette (10). Loaded with a force on its tip, the microneedle will bend with a deflection that is linearly proportional to the force. Although the lower limit of detecting the microneedle displacement is around 0.1 nm, the thermal motion of the microneedle tip compromised the force resolution to a value of ~0.6 pN for the softest microneedle (9). Besides, coating proteins of interest on the microneedle tip is not an easy task and monitoring the deflection of the microneedle usually needs the assistance of a plastic bead (10) or fluorescence microscopy (9). Nevertheless, since its invention, the microneedle technique has been refined and applied to many studies including measuring the force required to break the bond between two actin monomers (~110 pN), the force of a small number of myosin molecules interacting with a single actin filament (5-150 pN), the minimum force required for extracting a long membrane cylinder (tether) out of an erythrocyte (~50 pN), the adhesion energy between the lipid bilayer and cytoskeleton of erythrocytes (~60 pN/μm), and the bending stiffness of the erythrocyte membrane (~0.2 pN·μm) (9-12). More recently, the microneedle technique was used in studying the lifetime of E-selectin-sialyl-Lewis^x bonds under various loading rates of pulling forces (200–5000 pN/s) (13).

2. The Atomic Force Microscope

Like the microneedle technique, the AFM is also based upon the cantilever beam theory in solid mechanics (14). Here a silicon nitride cantilever instead of a glass microneedle is used as the force transducer. The deflection of the cantilever is monitored by shining a ray of laser light onto the back of the cantilever and by monitoring the angle change in its reflective ray with an array of photodiode. With this method, the position of the cantilever can be tracked as accurately as 1 Å, yielding a theoretical force resolution of 1 pN for a cantilever that has a stiffness of 10 pN/nm, which can be calibrated by recording its resonant frequency (14, 15). Combining both soft and stiff cantilevers, the AFM can measure forces in a wide range of 10-10^5

pN. With a feedback positioning mechanism and an ultra-soft cantilever (0.1 pN/nm), its force resolution can be improved to ~0.1 pN (16). Because of its convenience, versatility, and commercial availability, the AFM has spread rapidly around the world. A lot of progress has been made in the field of receptor-ligand interactions with the AFM. For example, it has been successfully applied to measuring the rupture force of a single avidin-biotin bond (160 pN), a proteoglycan bond (400 pN), and complementary strands of DNA (830 pN for 12 base pairs) (1, 17, 18). More recently, the AFM has been applied to unfolding individual titin immunoglobulin domains (150 to 300 pN for each domain at a pulling velocity of 1 μm/s), as well as individual tenascin fibronectin type III domains (an average of 137 pN at pulling velocities of 2 to 6 μm/s) (3, 19). Other than measuring forces, the AFM is capable of imaging solid surfaces with atomic resolution, but its resolution of imaging biological surfaces is not as high. One limitation to the AFM is that combining it with other microscopic techniques is difficult.

3. The Optical Tweezers

The optical or laser tweezers use a plastic bead that is trapped by a laser beam as the force transducer. If a force is imposed on the bead, the bead will attempt to escape the trap. Because of the bead displacement, the trap will impose an equal amount of force on the bead in the opposite direction due to the change in the laser momentum across the bead (20). The stiffness of the laser trap can be calibrated by creating a well-defined flow field around the bead or by analyzing the Brownian motion of the trapped bead (21, 22). The forces that can be measured with this technique are approximately from 1 pN to 100 pN. The optical tweezers have shown their great value in molecular and cellular biomechanics. For instance, the isometric force generated by a single kinesin molecule was found to be 1.9 pN, the peak force myosin exerts on actin was averaged at ~4 pN, and the minimum force required to extract a tether from neuronal growth cone membranes was measured to be about 8 pN (21, 23, 24). In addition, a coaxial dual-beam laser trap was very effective in revealing the hysteresis experienced by individual titin molecules during stretch and relaxation (2, 7). The advantage of the optical tweezers over other techniques is that it can manipulate living cells and large molecules or particles, but large forces cannot be measured or imposed unless multiple traps are utilized (25, 26).

4. The Magnetic Force Apparatus

In the magnetic force apparatus, a paramagnetic bead is used as the force transducer. A magnetic field is created around the bead to generate a magnetic force on the bead. This apparatus is calibrated by creating an aqueous flow field perpendicular to the magnetic field (27), by observing the thermal fluctuation of the transducer (5), or by attaching the bead to an inflated erythrocyte in a micropipette (28). Although it is the most sensitive technique, the magnetic force apparatus has not been adopted widely because even two beads from the same origin can have different susceptibility to the same magnetic field, making it difficult to operate. Nevertheless, this technique has been successfully applied to several seminal studies in single molecule/cell biomechanics, including the elasticity of a single natural or supercoiled DNA and the bending modulus of lipid membranes (5, 27, 28). To date, the lowest force that was ever applied to a single molecule was around 0.01 pN or 10 fN and it was done with the magnetic force apparatus. Had this capability of measuring femtonewton forces not existed, the details of the mechanical properties of DNA at low forces would not have been revealed (5, 6).

5. The Flow Chamber Assay

There are two versions of this technique: radial and parallel (29, 30). In both cases, the force application and calculation are exclusively based upon fluid mechanics. Suspensions of spherical cells or protein-coated beads are allowed to flow over a flat substrate where cells are cultured or proteins are immobilized. If molecular bonds can form between the substrate and the cell or bead, the cell or bead will rotate and form another contact with the substrate. Due to the flow of the medium, a force and a torque will be applied to the cell or bead. The force and torque can be calculated with fluid mechanics (31). In the meantime, the molecular bonds will also impose a force on the cell or bead. If the cell or bead is in mechanical equilibrium, this latter force can be calculated by equilibrating all the forces and torques on the cell or bead if the lengths of the molecules involved are known. If the cell surface is not smooth, which is the case for most cells, computational complications will arise. The flow chamber assay was first used in the study of antibody-antigen binding kinetics and later applied to P-, E-, and L-selectin kinetics (32-40).

6. The Biomembrane Force Probe

The BFP, which takes advantage of a micropipette manipulation system, is based upon membrane mechanics, a branch of solid mechanics. Its invention

was preceded by the study of detaching agglutinin-bonded erythrocytes with micropipette manipulation (41-43). The BFP can be used in measuring forces from 0.5 pN to 1000 pN with a wide range of loading rates from 0.1 pN/s to 10^6 pN/s (44). The probe is assembled with a ligand-coated microbead firmly attached to a pressurized capsule (an erythrocyte or a lipid vesicle), which is held by a micropipette (45, 46). The capsule acts as the force transducer and the displacement of the bead yields the magnitude of the force. The stiffness of the probe is proportional to the surface tension of the capsule. Different surface tensions, achieved by applying different suction pressures to the capsule, produce different force sensitivity (1 pN/μm to 10^4 pN/μm). The major achievement of the BFP was the determination of the energy landscape of breaking a single avidin-biotin bond and the force spectrum of extracting a lipid molecule from the lipid bilayer of vesicles (44, 47). However, the BFP requires a specialized orthogonal microscope and a sophisticated protein coating procedure for its operation, so it has only been established in a few laboratories around the world.

7. The Micropipette Aspiration Technique

Like the BFP, the MAT also takes advantage of a micropipette manipulation system, but it is based upon fluid mechanics. The only requirement for the force transducer of the MAT is its sphericity, *i.e.*, either a spherical cell like a human neutrophil or a spherical object like a latex bead can be employed as the force transducer. The force transducer is placed inside a micropipette whose diameter is almost the same as the diameter of the transducer as shown in Fig. 1 where an antibody-coated bead fits snugly and moves freely inside the left micropipette. A precise aspiration pressure (Δp) can be applied across the bead and the pulling force imposed on the bead by the fluid flow, F, can be calculated with the following equation (48):

$$F = \pi R_p^2 \Delta p \left(1 - \frac{4}{3}\bar{\varepsilon}\right)\left(1 - \frac{U_t}{U_f}\right) \qquad (1)$$

where R_p is the radius of the micropipette, U_t is the velocity of the bead after adhesion, and U_f is the velocity of the bead when it is moving freely under the same pressure Δp, and

$$\bar{\varepsilon} = (R_p - R_b)/R_p \qquad (2)$$

where R_b is the radius of the bead. The first correction term in Eq. 1 accounts for the gap between the bead and micropipette, while the second correction term accounts for the bead motion. After adhering to the cell, the bead can move under the pulling force due to the formation of tethers in the case shown in Figure 1 (4, 49). Under other circumstances like the deformation of molecules, the bead could also move after adhering to the cell. If U_t is zero or another constant, a constant force can be readily applied. If a bead that has a radius of 2 μm is used as the force transducer, a theoretical force resolution of 0.1 pN can be achieved with a pressure resolution of 0.01 pN/μm^2. However, it is difficult to use a bead smaller than 2 μm as the force transducer because whether the bead fits snugly inside the micropipette will be difficult to visualize and large pressure uncertainties can occur. With a spherical neutrophil as the force transducer, it was found that a human neutrophil microvillus would be extended like a spring with a spring constant of ~43 pN/μm if a pulling force less than 45 pN was applied. If a force larger than 45 pN was applied, a tether would be pulled out at a velocity proportional to the force (4). It was also found that single neutrophil receptor anchoring strengths varied among three clusters of receptors (CD62L, CD18, and CD45) (50). With a latex bead as the force transducer, the MAT was used in studying the expression and kinetics of E-selectin on endothelial cells and tether formation from treated human neutrophils (49, 51). The advantage of the MAT over other techniques is that a spherical cell can be used directly as the force transducer and very large forces like 100 nN can be imposed by applying large aspiration pressures (48).

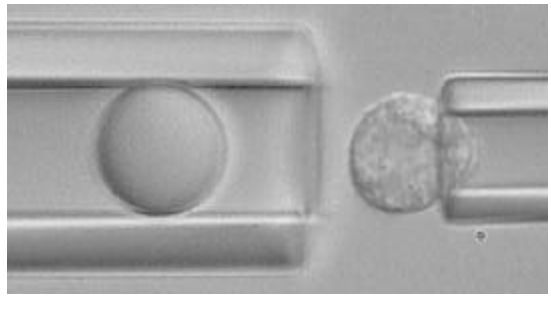

Figure 1 A microscopic view of the micropipette aspiration technique. Here the adhesion between a protein-coated latex bead (left) and a human neutrophil (right) is being probed with the MAT.

With a slight modification, the MAT can be extended to imposing forces at a femtonewton level. If the bead shown in Figure 1 is placed outside the left micropipette after adhering to the cell, the pulling force (f) imposed on the bead by the same aspiration pressure will be much smaller than the one calculated with Eq. 1. The magnitude of f can be calculated with computational fluid mechanics. Assume that the distance between the bead and micropipette opening is 4 μm, the maximum fluid velocity in the micropipette is 10 μm/s (corresponding to an

aspiration pressure of 1.25 pN/μm^2 for a micropipette that has an equivalent length of 500 μm), the radius of the cell is 4 μm, the thickness of the micropipette wall is 1 μm, the radius of the right micropipette is 2 μm, R_p = 4 μm, and R_b = 4 μm, then f = 0.222 pN or 222 fN (52). Forces smaller than 222 fN can be imposed with smaller micropipettes, beads, or aspiration pressures.

8. Other Techniques

The surface force apparatus is another useful technique for probing molecular interactions, but its force resolution is only around 10 nN (53, 54). Therefore, only the average behavior of multiple interactions can be measured. Two other techniques that are based upon fluid mechanics exist, but they are both specific to certain problems (55, 56).

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