Quantification of Cell Adhesion Strength using Energy Dissipation from Quartz Microbalance with Dissipation Monitoring

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***Abstract*— We propose a mechanical model that describes the energy dissipation process in the probing of cell adhesion using quartz crystal microbalance with dissipation monitoring (QCM-D). The model considers the QCM-D disk as a harmonic oscillator and the friction between the disk and the cell is modeled as molecular bond rupturing and the fluidic slip at the interface. The bond formation and rupture events are governed by relative motion between the sensor disk and the cell membrane. We consider this interaction as the main energy dissipation channel for the oscillator, as the dynamic molecular bond rupture and the viscous damping of the trapped liquid at the cell/disk interfacial layer contribute to the most energy loss during the harmonic oscillation. The energy loss due to the frictional slip of the stress fiber/cytoplasm is insignificant compared with the bond rupture. At high bond number conditions, the energy dissipation will be dominated by the bond rupture events at the focal adhesion, and bond number and the size of focal adhesion are linearly related to the energy dissipation factors. These findings can serve as an analytical tool for QCM-D based cell adhesion assays.**

I. Introduction

The quartz crystal microbalance with dissipation monitoring (QCM-D) is an acoustic biosensor that measures near surface changes by recording the mechanical oscillation of a quartz crystal. The shear mode oscillation is produced by an alternating voltage applied to a thin quartz crystal sandwiched between a pair of electrodes. The piezoelectric property of the quartz crystal results in a displacement difference between the top and the bottom electrodes, and the displacement shift applies a shear stress at the measuring surface. QCM-D device can detect subtle changes to cell adhesions, such as the remodeling of cytoskeleton and the assembly/disassembly of focal adhesion complexes, under different physiopathological conditions [1-3]. For instance, when suspended cells start to attach and proliferate on the sensor disk, the spreading activities can be monitored in real time from changes in frequency and energy dissipation [4]. Once these changes are captured, the interpretation of frequency shifts and energy dissipation factor variations in terms of the biophysics of cell adhesion can establish the measured data as an effective marker for cell adhesion [5-8].

To characterize the related cell property, a physical model that describes the experimental condition and take into consideration the biophysical interactions between the sensor and the cells needs to be established. Most of the current models to date are based on the equivalent circuit and impedance analysis methods [9-11]. A commonly accepted model [12] uses a continuum mechanics approach and modeled the propagation process of the mechanical wave generated by the sensor oscillation through a viscoelastic material covered by a Newtonian fluid. By setting up appropriate boundary conditions, the change of frequency and energy dissipation factor can be expressed in terms of the physical properties of the viscoelastic thin film and the fluid. These modeling approaches along with others reviewed in [9], though popular and easy to use, treat the cells as a homogeneous material and neglect the composite nature of the cell structure. However, more evidence points towards the focal adhesion complex as one of the main units that physically interact with the sensor disk during the oscillation [13] and there exists coupled multi-dimensional energy dissipation sources at the fluid-solid interface. The current models failed to take into consideration of these physical interactions that governs the energy dissipative processes during the measurement. Thus, a mechanical model that uses the mechanical oscillation as the measurement energy source and models the cell/substrate interaction in detailed fashion down to the molecular level is required to better understand the measured QCM-D data.

We have previously shown a strong correlation between the focal adhesion and the measured energy dissipation factor [13, 14]. We believe the frictional bond slip between the integrin and extracellular matrix (ECM), the viscous damping between basal membrane and the trapped liquid between the basal membrane and the substrate, as well as the frictional slip between the focal adhesion complex and the stress fibers within the cell are all sources of energy dissipation, and they contribute in different manners and magnitudes during each measurement cycle. Thus, we propose a mechanical model based on the dynamics of the oscillation sensor disk as well as the kinetics of the focal adhesion and stress fiber. The model will consider these energy dissipative mechanisms and quantify their contributions, and ultimately serve as an analytical tool for QCM-D related cell adhesion assays.

II. Methods

A. Oscillator modeling

The oscillating sensor disk experiences the friction from the attached cell and the fluid which damps the oscillation and slows down the movement. Modern tribology normally theorizes that friction at the microscopic level stems from the formation and rupture of molecular bonds [15, 16]. A simple model containing two plates with relative motion connected by molecular bonds has been developed to address the frictional energy dissipation through bond rupture and viscous damping [17]. Since the energy dissipation through the viscous damping at the liquid-solid interface can be easily comprehended [18, 19]; while the model mainly focused on the energy dissipative process through the stick-slip motion of molecular bonds [20].

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Mechanical forces exerted from the oscillation break the energy equilibrium of bond formation and dissociation. The bond length and rupturing probability become dependent or a function of the applied force; Therefore, the loading forces from the oscillation lower the energy barrier of the unbinding process and increase the dissociation probability and ultimately dissipate the kinetic energy of the oscillation. This was described by the classic Bell's model of bond kinetics under mechanical influences [21, 22]. Base on the friction model and combined with the Bell's model of molecular bond kinetics, we designed a mechanical model to simulate the QCM-D measurement of cell adhesion.

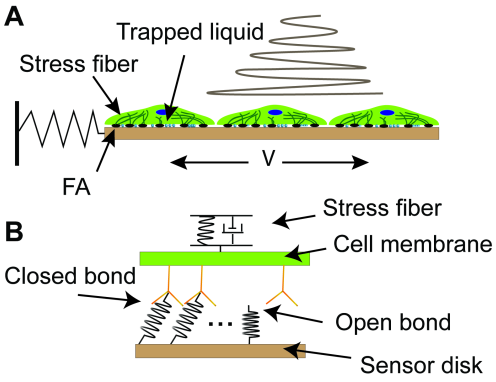

Figure 1. The energy dissipation model for QCM-D/cell adhesion interaction. (A) An alternating voltage applied to electrodes induces relative displacement between the top and the bottom surfaces, thus generates a periodic shear motion on the interface between the cell and the disk. (B) The mechanical structural model describes the sensor disk as a harmonic oscillator with damping contributions from the trapping liquid between the disk and the physical interactions between cell and sensor disk via focal adhesion

The proposed mechanical model treats the quartz crystal as a one-dimensional harmonic oscillator and the oscillation is maintained by a spring with a spring constant *k*. The model is illustrated in Fig. 1A. The sensor disk is anchored to a spring with an initial displacement as the energy input. The physical interaction between the sensor disk and the cell, i.e. friction in a generalized description, is described by the bond forces (F_b) at the interfacial layer from the integrin-ECM adhesion, and the viscous damping forces ($\eta_l \dot{X}$) from the liquid trapped between the basal membrane and the sensor disk. Thus, the dynamics of the disk oscillation can be defined as:

$$m\ddot{X} + \eta_l \dot{X} + F_b + kX = 0, \tag{1}$$

where m is the mass of the disk and η_l is the viscous damping coefficient; X is the index of the disk with horizontal motion; k is the spring constant of the anchoring spring. Therefore, $\eta_l \dot{X}$ represents damping from the intrinsic decay and by the trapped fluid. kX is the force in the spring and $m\ddot{X}$ denotes the inertial force resulted from the acceleration of the sensor disk. The energy loss of the sensor disk in a damped oscillation can be described as energy dissipation factor (D), which is defined as the ratio between the energy loss and the energy input:

$$D = \frac{1}{2\pi}\frac{E_{\text{in}} - E_{\text{out}}}{E_{\text{in}}}, \tag{2}$$

where E_{in} is the input energy and E_{out} is the output energy. The input energy is the initial total energy of the system defined as $E_{\text{in}} = \frac{1}{2}kA_0^2$; while the output energy (the sum of kinetic energy of sensor and potential energy of spring) is the current total energy of the system defined as: $E_{\text{out}} = \frac{1}{2}M\dot{X}^2 + \frac{1}{2}KX^2$.

B. Bond kinetics modeling

The friction force contributing to the energy dissipation of the oscillation from focal adhesion bonds is the sum of forces in each individual bond projected in the motion direction, X (Fig. 1B):

$$F_b = \sum_{i=1}^{N} q_i f_i^X, \tag{3}$$

where F_b is the total force, i is the bond index and N is the number of bonds in the focal adhesion complex; f_i^X is the bond force in the ith bond projected in the X direction; q_i is the state of the bond with $q_i = \begin{cases} 0 & \text{open bond} \\ 1 & \text{closed bond} \end{cases}$ [23]. If a bond is closed, the force it sustains is defined according to Hook's law: $f_i = \kappa_b[l_i - l^0]$, where κ_b is the spring constant of the bond, with the projection in the X direction $f_i^x = f_i x_i / l_i$; l_i is the bond length and x_i is its projection in the X direction. The bond movement ($\dot{x}_i$) can be defined with respect to the substrate movement ($\dot{X}$) as:

$$\dot{x}_i = q_i\dot{X} - \lambda(1 - q_i)x_i \tag{4}$$

where λ is the bond retraction rate when it is ruptured. According to the Bell model, applied load reduces the energy barrier of bond dissociation and increases the rate of bond rupture, and thus redefines the initial bond dissociation rate:

$$k_{\text{off}}(l_i) = k_{off}^0 \, exp\left(\frac{f_i \Delta l_i}{k_B T}\right), \tag{5}$$

where f_i is the bond force; Δl_i is bond extension; k_B is the Boltzmann constant and T is the absolute temperature. k_{off}^0 is the initial rate constant for bond dissociation [24]. On the other hand, bond formation rate, k_{on}, can be redefined by the loading rate:

$$k_{on} = \begin{cases} k_{on}^0 & v \le v_c \\ 0 & v > v_c \end{cases} \tag{6}$$

where k_{on}^0 is the initial loading rate and v is the velocity of the substrate. Equation (6) states that bond formation is prohibited at higher loading rate, specifically higher than v_c [25].

C. Stress fiber and energy dissipation

The viscous damping at stress fiber and the cytoplasm is represented by a lumped model using the viscoelastic Voigt element, i.e., a spring-dashpot combination in parallel, as illustrated in Fig. 1B. Stress fibers are connected to the focal adhesion via a central plaque, hence the force balance in the stress fiber with respect to the bond forces is defined as [26]:

$$\sum_{i=1}^{N} q_i f_i = k_{sf}\Delta l_{sf} + \mu_{sf}\Delta\dot{l}_{sf} \tag{7}$$

where k_{sf} and μ_{sf} are the elastic and damping coefficients of the stress fiber; Δl_{sf} and $\Delta\dot{l}_{sf}$ are the extension and extension rate of the stress fiber, respectively.

The energy dissipation of the mechanical model mainly comes from these two channels: the friction at the interface of cell-disk contact (E_a) and the viscous damping of the stress fiber ($E_d^h = \sum_{i=1}^{N} q_i f_i \Delta l_{sf}$). The cell-disk friction at the microscopic level also comes with two components ($E_a = E_b + E_v$): the rupture of adhesion bonds between the cell and the sensor disk when bonds switch from closed to open states ($E_b^h = \sum_{i=1}^{N} q_h(1-q_h) f_i \Delta l_i$) [27] and the viscous damping through the trapped liquid at the interface ($E_v = \int_{jh}^{(j+1)h} \eta_l \dot{X}^2 \, dt$).

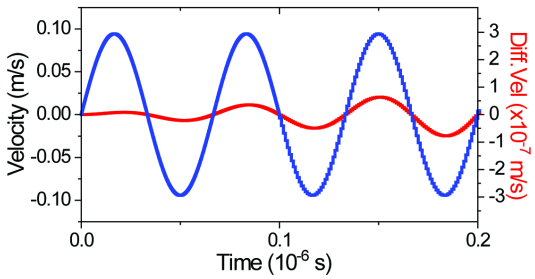

Figure 2. Dynamics of the sensor disk oscillation damped by cell-disk interface friction and intracellular friction, and its interaction with cell adhesion bonds. Differences of velocity compared with an un-damped system (red) Velocity changes during each oscillation cycle (blue).

III. Results

The mechanical model was simulated using a customized MATLAB program with a simulation step of 10^{-12} s. The velocity profile of the sensor disk (Fig. 2, blue) and its difference with an un-damped system (Fig. 2, red) shows that the energy of the whole system is being dissipated. The spontaneous bond association and rupture processes are strongly influenced by the relative motion between the sensor disk and the cell [23, 26]. The rate of bond formation is modulated by the velocity profile of the sensor oscillation.

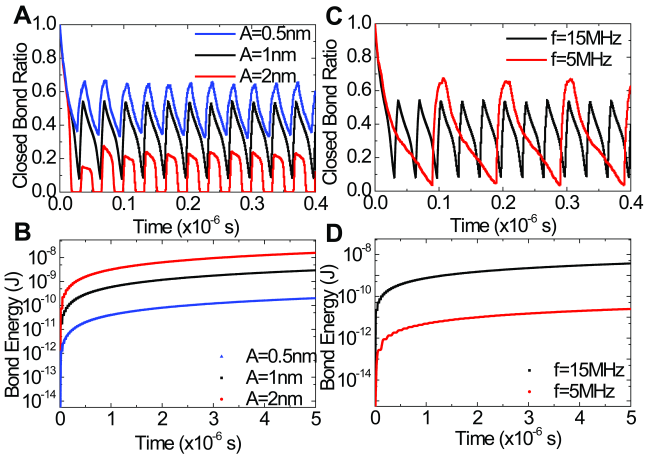

Figure 3. Influence of oscillation on bond kinetics and bond energy loss. (A) The closed bond ratio and bond energy loss with respect to different maximum oscillation amplitudes (B) Total energy loss with respect to different maximum oscillation amplitudes. (C) The closed bond ratio and bond energy loss with respect to different oscillation frequencies (D) Energy loss due to bond rupture with respect to different frequencies.

For the kinetics of the bond formation and rupture, we performed parametric study. As shown in Fig. 3, when the relative velocity between the sensor disk and the cell exceeds the critical loading rate v_c, bond formation stops and the closed bond ratio υ is dominated by bond rupture events as indicated by gradual decrease in υ from a peak value (υ_{max}) to the minimum (υ_{min}). Peak closed bond ratio (υ_{max}) is influenced by the maximum amplitude (A_0) and frequency (ω) of the sensor oscillation. Larger A_0 results in longer bond extension and thus increases bond dissociation rate exponentially according to Bell model (Fig. 3A). With less bond dissociation, the energy loss due to bond rupture events will be about two to three orders apart (Fig. 3B). Similarly, higher oscillation frequency not only increases the number of cycles for bond formation and dissociation, but also reduces the amount of time for v to reach v_c and thus lowers the bond formation duration and ultimately suppresses υ_{max} (Fig. 3C). Changing from a fundamental frequency of 5 MHz to third overtone 15 MHz increases the energy cost from bond rupture by about one folds (Fig. 3D).

There are three main channels of energy dissipation in the mechanical model: friction at the cell-sensor disk interface is characterized by the dynamic molecular bond rupture events and the viscous damping caused by the trapping fluid dissipate energy; intracellular frication is through the lumped viscous damping of the stress fiber and cytoplasmic material. These three energy dissipation channels consumed the kinetic energy of the oscillating disk. At lower bond numbers, of the three energy dissipation channels, the stress fiber dissipates the least amount of energy with average energy loss per oscillation cycle eight orders of magnitude lower than the viscous damping of the trapping liquid at the interface. The bond rupture energy dissipation is two orders of magnitude less than the trapped liquid energy dissipation. As the number of bond increase, energy dissipation by the viscous damping of trapping liquid remains in the same level; since bonds do not possess volume, the change of trapping fluid at the cell-disk interface is essentially negligible, thus the energy loss depends entirely on the velocity profile at each oscillation cycle. On the other hand, energy dissipation through the synchronized bond rupture events increased almost linearly with respect to the bond number (Fig. 4A).

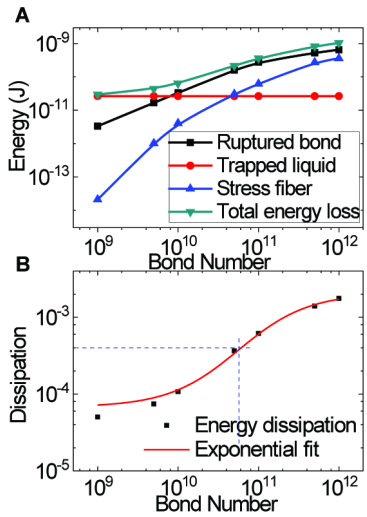

Figure 4. Contribution from different energy dissipation channels and energy dissipation factor with respect to bond number. (A) Contributions from bond rupture, trapping liquid friction and stress fiber viscous damping in energy loss at different bond numbers. (B) Energy dissipation factor increases with larger bond number. The simulation data was fitted with an exponential curve.

Viscous damping in the cytoplasm between the stress fibers and the focal adhesion complexes dissipates negligible amount of energy at lower bond numbers; as bond number increases, the total bond force applied onto stress fiber increases proportionally, which results in a linear increase of energy dissipation with respect to bond number. This increasing trend saturates when bond number reaches around 10^{12}. At this bond number, energy dissipation will be dominated by cell-ECM adhesion related energy loss. The energy dissipation factor *D* increases with bond number. It grows exponentially as bond number crosses 10^{12} threshold and saturates to one when bond number is too large and dissipates energy fast (Fig. 4B). The simulation also showed that the sum of energy dissipation from the three channels equal the total kinetic energy loss.

IV. Conclusion

In conclusion, we designed a mechanical model based on the physical interaction between the QCM-D disk and the cell focal adhesion. The proposed model considers the sensor crystal as a harmonic oscillator and the friction at the cell/disk interface due to the dynamic molecular bond rupture as well as the viscous damping at stress fiber/cytoplasm causes the kinetic energy loss during each oscillation cycle. We demonstrated that the QCM-D energy dissipation comes mainly from the cell focal adhesion and sensor interaction and the stress fiber damping, while the viscous damping caused by the trapping fluid at the interface contributes insignificantly. The energy dissipation almost linearly correlates with the bond number and thus the focal adhesion size. The finding confirms the experimental results obtained earlier with the similar correlation. The proposed model, different from present models which are overwhelmingly relied on equivalent circuit method, emphasizes the importance of the physical interaction between the focal adhesion and the sensor disk, and its role in the energy dissipation process of sensor oscillation.

It is worth mentioning that the cell-cell adhesion was not considered in the model, as we perceive that the cell-cell interaction needs to work through the cell-ECM adhesion, and thus the force contribution from cell-cell adhesion is reflected in the cell-ECM adhesion measurement. If this model is proved valid, this model and its simulation result can serve as an analytical tool for QCM-D based measurements of cell adhesion in a label-free and real-time manner.

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