The 7th Annual IEEE International Conference on
Cyber Technology in Automation, Control and Intelligent Systems
July 31-August 4, 2017, Hawaii, USA

Modeling the Viscoelastic Properties of Living Cells by Considering the Cantilever Effect

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***Abstract*—Cells are the basic component units of organisms, and reflect the physiological state of creatures. The mechanical properties of a cell reflect the cytoskeletal structure and composition and are closely related to the cellular biological functions and physiological activities. Therefore, modeling the mechanical properties of single cells provides the basis for analyzing and controlling the cellular state. Previously, we developed a dynamical model with cellular viscoelasticity properties as the system parameters to describe the stress-relaxation phenomenon of a single cell indented by an atomic force microscope (AFM). However, in this model, the effect of the AFM cantilever was neglected and the viscoelastic properties of both the cell and the AFM cantilever are considered as a whole in the model. In this study, we modified the model by considering the AFM cantilever as an independent factor and the elasticity of AFM cantilever and the viscoelasticity of cells are decoupled. The modified model can characterize the mechanical properties of single cells without the influence of AFM cantilever.**

Keywords—single cell; dynamic model; mechanical properties; atomic force microscopy; viscoelasticity

I. BACKGROUND

Cells are the basic component units of organisms, and reflect the physiological state of creatures. The mechanical properties of a cell are related to the cellular behavior, such as cell growth, division, differentiation, and play a significant role in the regulation of cell physiology [1]. The structure and composition of the cytoskeleton of a cell can be reflected by its mechanical properties. The variation in the mechanical properties of cells is associated with the emergence and development of human disease [2]. Cellular mechanical information can be utilized as a label-free biomarker for cell recognition [3], early diagnosis of disease and drug efficacy evaluation [4]. Therefore, it is important to measure and quantitatively describe the mechanical properties of a single cell using a mathematical model. However, a cell is a complex network that contains thousands of overlapping signaling pathways [3]. Traditional methods for describing the dynamics of this network are extremely complicated [4]. Therefore, it is difficult to analyze the global properties of cells via the underlying mechanism, let alone to determine how to control them.

The Hertz model [5] is a widely used model to describe the relationship between the force and indentation depth. The Young's modulus of cells can be statistically calculated using the Hertz model for the force-indentation curves. However, the Hertz model has some issues that need to be addressed. For instance, the Hertz modified model assumes that the measured materials are linearly elastic and isotropic, which in general does not holds for cells. On the other hand, the Hertz model can reflect only the elasticity of the cell. Using a three-element standard solid model, J. R. Dutcher et al. extracted the elastic and viscous properties of the bacterial cell envelope separately from the time-dependent creep deformation curve, which resulted from a constant force [6]. Similarly, A. Yango et al. applied a linear standard solid model to calculate the elastic and viscous properties of soft materials from the creep response to the loading and unloading steps during the stress-relaxation phase of AFM indentation [7]. In these two methods, the elastic and viscous properties are decoupled from the indentation force curves with the standard linear solid model; however, both methods assume that the soft materials are a first-order system, which ignores the high orders of the complex viscoelasticity properties of living cells. We have previously demonstrated a general Maxwell model without a pre-assuming order for describing the mechanical viscoelastic properties, and the system order and parameters can be determined by using the system identification method from the experimental data in the indentation process [8]. In this work, system order and parameters were identified using the Hankel matrix method and least squares method, respectively. To validate the dynamical model for cell deformation behaviors under a constant indentation depth, we performed indentation experiments using four types of cells, namely MCF-7, HEK-293, L-929 and Neuro-2A cells. For all the cells, the dynamical system was determined to be second order, which means the mechanical properties of a single cell can be modeled as a second-order linear system, and for each system, five parameters could to be identified by the stress-relaxation curves. Then these five-parameter tuples can be used as biomarkers to classify different types of cells. However, in this model, the effect of the AFM cantilever was neglected and the viscoelastic properties of both the cell and the AFM cantilever are considered as a whole in the model. In this study, we modified the model by considering the AFM cantilever as an independent factor and the elasticity of AFM cantilever and the viscoelasticity of cells are decoupled. The modified model can characterize the mechanical properties of single cells without the influence of AFM cantilever.

II. MATERIALS AND METHODS

A. Cell Preparation

The cell lines used in this study were obtained from the Institute Pasteur of Shanghai, Chinese Academy of Sciences (Shanghai). MCF-7 cells (human breast cancer cell line), L-929 cells (mouse fibroblast cell line), Neuro-2a cells (Mus musculus

978-1-5386-0490-8/17/$31.00 © 2017 IEEE

brain neuroblastoma cell line) and HEK-293 cells (human embryonic kidney cell line) were cultured in RPMI-1640 (Thermo Scientific HyClone, USA) containing 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37°C (5% CO2). These four types of cells were cultured in Petri dishes. The diameter of the Petri dishes we used is 60 mm, and the cell concentration is about 1.3×10^6 per square centimeter. The cells were cultured for 24 h before experiments. The same experiments were performed with different batches of cells. All the AFM experiments in this study were performed in culture medium. The experiments were conducted at room temperature.

B. *Indentation Process*

In this study, we used a Bioscope Catalyst AFM (Bruker, USA) and an inverted microscope (Nikon, Japan). The type of AFM probe used in this study was MLCT (Bruker, USA), and the nominal spring constant of the cantilever used was 0.01 N/m. We used the thermal tune to calculate the spring constant of the cantilever. And the actual value of the spring constant k_p is about 0.08 N/m.

The standard AFM indentation experiments were used to obtain the stress-relaxation curves of cells. In general, the indentation experiments can be divided into three phases, approach, stress-relaxation, and retraction. As shown is Fig.1, in the approaching phase, the position of piezoelectric actuator (PZT) dropped with a constant speed and the cantilever deflected due to the contact with the cell. In the stress-relaxation phase, the PZT position was a constant, and the cell continued deforming due to the stress-relaxation. In the retraction phase, the PZT position rose and the cell recovered from the deformation. In this study, the speed of PZT in the indentation experiments was 4 μm/s during both the approach and retraction phases. The stress-relaxation time is 6 seconds, which can keep the stress-relaxation curves tend to steady. The maximum distance of cantilever displacement is 1 μm.

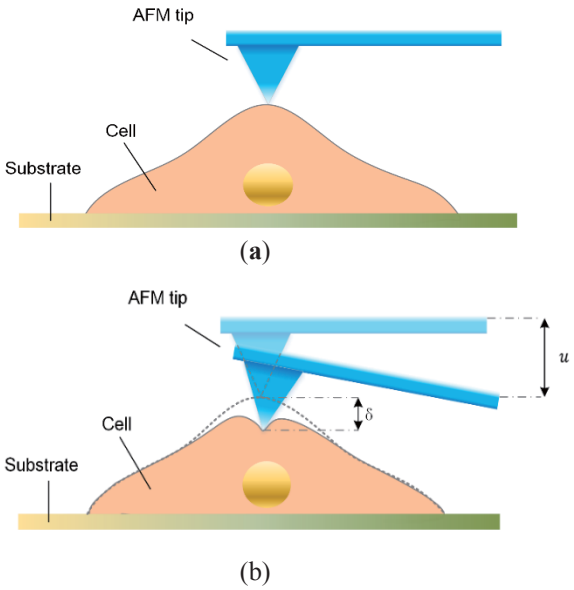

Fig.1 Illustration of the AFM indentation experiment and the experimental curve from one entire indentation process. (a) (b) Schematic diagram of indentation applied using an AFM probe tip on a single cell.

C. *Dynamical Modeling of the Viscoelastic Properties of a Single Cell with the cantilever*

In this study, system science was used to model the dynamical mechanical behavior of a single cell with its viscoelastic properties as the system parameters by considering the effect of the AFM cantilever, based on the input (stimuli) and corresponding output (responses) instead of the interior structure of the cells. A general Maxwell model with an extra independent factor of spring k_p is used to describe the dynamic behavior of cells with the viscoelasticity of cells and the elasticity of AFM cantilever as the system parameters , as shown in Fig. 2. k_p represents the elasticity of the AFM cantilever. In this study, the spring constant of the cantilever is determined by thermal tune, which we have already mentioned before, and the AFM PZT z-position during the indentation is used as the input signal $u(t)$, and the measured force is used as the output response $y(t)$ of the cell system. The rest part of the model is a general Maxwell model for viscoelastic materials, which contains a spring k_0 and n parallel spring-damping paths. This general Maxwell model part represents the viscoelasticity of the cell [9]. It is noting that the number of parallel spring-damping paths, or the order of the spring-damping model, is not pre-assumed and will determined by using the system identification method and experimental data in the indentation process. The state-space equations of the model are as follows.

$$\begin{cases}\dot{x}(t) = Ax(t) + Bu(t)\\ y(t) = Cx(t) + Du(t)\end{cases} \tag{1}$$

Where,

$$A=\begin{bmatrix}\frac{-k_1(\Sigma-k_1)}{b_1\Sigma} & \frac{k_1k_2}{b_1\Sigma} & \cdots & \frac{k_1k_n}{b_1\Sigma}\\ \frac{k_2k_1}{b_2\Sigma} & \frac{-k_2(\Sigma-k_2)}{b_2\Sigma} & \cdots & \frac{k_2k_n}{b_2\Sigma}\\ \vdots & \vdots & \ddots & \vdots\\ \frac{k_nk_1}{b_n\Sigma} & \frac{k_nk_2}{b_n\Sigma} & \cdots & \frac{-k_n(\Sigma-k_n)}{b_n\Sigma}\end{bmatrix}, B=\begin{bmatrix}\frac{k_1k_p}{b_1\Sigma}\\ \frac{k_2k_p}{b_2\Sigma}\\ \vdots\\ \frac{k_nk_p}{b_n\Sigma}\end{bmatrix}$$

$$C=\begin{bmatrix}\frac{-k_1k_p}{\Sigma} & \frac{-k_2k_p}{\Sigma} & \cdots & \frac{-k_nk_p}{\Sigma}\end{bmatrix}, D=\begin{bmatrix}\frac{k_p(\Sigma-k_p)}{\Sigma}\end{bmatrix}$$

$\Sigma = k_p + k_0 + k_1 + \cdots + k_n$, $u(t)$ is the system input, or the PZT movement, which is not equal to the distance that the cell was pushed down in the indentation process. $y(t)$ is the system output, i.e., the force that AFM measured. $x(t) = [x_1(t), \ldots, x_n(t)]^T$ is a n-dimensional vector which is called the state variable of the system. $x_i(t)$ represents the movement distance of the point between the spring and damper in the ith path, k_p is the spring constant of the cantilever, which can be pre-determined by thermal tune, and k_i and b_i are the elastic and viscous parameters of the corresponding springs and dampers, respectively. The state $x(t)$ are closely related to the cell deformation. In particular, the system output can be seen as a step response when the input $u(t) = \delta(t)$, where $\delta(t)$ is the unit step function. Thus, the signals during the stress-relaxation phase can be regarded as the step response to the constant input, the PZT distance, to the system, and we could obtain the impulse response series by calculating the difference between every two adjacent points in the step response sequence. This model

illustrates that the elasticity and viscosity of cells can be represented by the multiple parameters k_i and b_i.

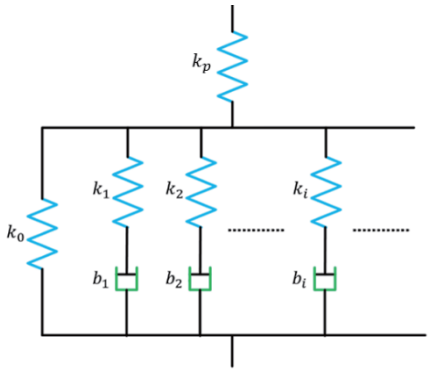

Fig.2 Schematic diagram of a general Maxwell model with an independent spring k_p

D. Order and Parameters Identification

In this section, the order and parameters of the cell system need to be determined from the input and output data. In this study, we used the Hankel matrix method to determine the order of the linear system. For linear systems, the Hankel matrix method is a classical approach for determining the order [25]. In this method, Hankel matrices are built from the impulse response sequence of the system, and the order of the system is actually the rank of the Hankel matrices. The criterion for identifying a system order using Hankel matrices is described in the following lemma.

Lemma 1. Let $\{g(i)|i = 1, 2, \dots, L\}$ be the impulse response sequence of a linear system. Hankel matrices can be constructed as follows:

$$H(l,k) = \begin{bmatrix} g(k) & g(k+1) & \cdots & g(k+l-1) \\ g(k+1) & g(k+2) & & g(k+l) \\ \vdots & & \ddots & \vdots \\ g(k+l-1) & g(k+l) & \cdots & g(k+2l-1) \end{bmatrix}$$

where l determines the dimension of the Hankel matrix, and k is any integer between 1 and $L-2l+2$. The order of the system is equal to the rank n_0 of the Hankel matrices if

$$rank[H(l,k)] = n_0, \ for\ all\ l \geq n_0, \forall k \qquad (2)$$

This criterion works perfectly for noise-free data. In general, the impulse response sequence includes noises, so the rank of the Hankel matrix may be not equal to n_0 exactly, even when $l \geq n_0$. Therefore, an equivalent criterion using the average ratio of the determinant of Hankel matrix D_l is used to evaluate the singularity of the Hankel matrices and order of the dynamical systems, where

$$\mathrm{D}_l = \frac{\frac{1}{L-2l+2}\sum_{k=1}^{L-2l+2} \det[H(l,k)]}{\frac{1}{L-2l}\sum_{k=1}^{L-2l} \det[H(l,k)]}$$

in which l is the dimension of the Hankel matrices and is not equal to 1. The determinant D_l grows as l increases if $l < n_0$ and then decays for $l > n_0$, and reaches a maximum at $l = n_0$. Therefore, the value of l at which D_l reaches the maximum value can be considered to be the order of the system. In this study, we used D_l to evaluate the order of the cell system.

In practice, the impulse response sequence of a dynamical system can be obtained by calculating the difference between every two adjacent points in the step response sequence of the system, i.e., $g(i) = y(i+1) - y(i), i = 1,2, \dots, L$, where $g(i)$ is the ith element in the impulse response sequence, and $y(i)$ is the ith element in the step response sequence. As we mentioned above, the stress-relaxation curves can be regarded as system's step responses, therefore we can obtain the impulse response sequence from the recorded force curves and construct the Hankel matrices. Once the order of the dynamic system of a single cell is determined, the parameters of the system can be easily determined by comparing the coefficients of transfer function, which we will discuss in the next section.

III. Results and Discussion

To validate the dynamical model for cell deformation behaviors under a constant indentation depth, we performed indentation experiments using four types of cells, namely MCF-7, HEK-293, L-929 and Neuro-2A cells, and the corresponding stress-relaxation force curves were collected for system order and parameter analysis. In our study, for each type of cells, the stress-relaxation curve remains steady after 4 seconds. The indentation depth is about 0.6~0.7 μm, and the maximum interaction force between the tip and the cell is less than 5 nN. In this study, only the force curves during the stress-relaxation phase were used to validate the dynamical model and to identified the system parameters. To reduce the noisy effect in the measurement, a low-pass filter with a 10-Hz cut-off frequency was used to smooth the force curves for further analysis, as shown in Fig. 3(b).

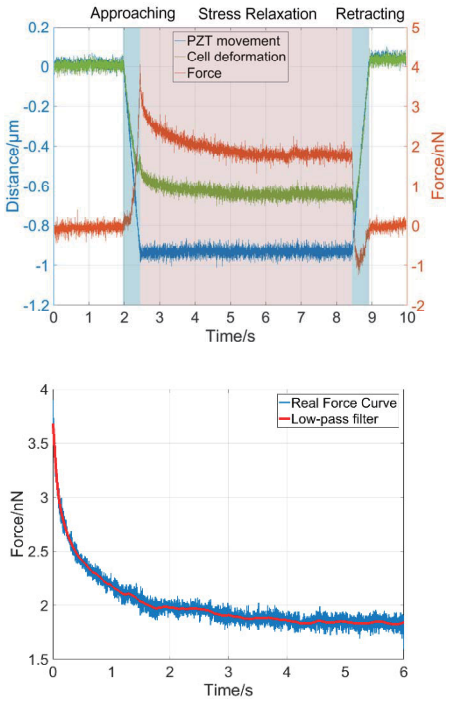

Fig.3 Illustration of the experimental curve from one entire indentation process. (a) The entire indentation process. (b) Original force curve (blue) in the stress-relaxation phase and smoothed curve using a low-pass filter.

For all the cells, the dynamical system was determined to be second order. The system matrices $\Phi = [\phi_{ij}]$ of the second-order state-space equations of the cells are as follows.

$$\Phi = \begin{bmatrix} A & B \\ C & D \end{bmatrix} = \begin{bmatrix} \frac{-k_1(\Sigma - k_1)}{b_1\Sigma} & \frac{k_1 k_2}{b_1\Sigma} & \frac{k_1 k_p}{b_1\Sigma} \\ \frac{k_1 k_2}{b_2\Sigma} & \frac{-k_2(\Sigma - k_2)}{b_2\Sigma} & \frac{k_2 k_p}{b_2\Sigma} \\ \frac{-k_1 k_p}{\Sigma} & \frac{-k_2 k_p}{\Sigma} & \frac{k_p(\Sigma - k_p)}{\Sigma} \end{bmatrix}$$

The corresponding transfer function of the system is

$$G(s) = \frac{\beta_2 s^2 + \beta_1 s + \beta_0}{s^2 + \alpha_1 s + \alpha_0}$$

where, $\alpha_0 = \det[A]$, $\alpha_1 = -\mathrm{tr}[A]$, $\beta_0 = \det[\Phi]$, $\beta_1 = -\mathrm{tr}[A]D + CB$, $\beta_2 = D$. $\det[\cdot]$ represents the determinant of a matrix, and $\mathrm{tr}[\cdot]$ represents the trace of a matrix.

The transfer function of a cellular system can be identified using the input and output curves by the System Identification Toolbox of Matlab. Once the transfer function of the cellular system is determined, we can calculate the parameters $\theta = [k_0, k_1, b_1, k_2, b_2]$ by comparing the coefficients of the transfer function. In particular, we can obtain the parameters $\theta = [k_0, k_1, b_1, k_2, b_2]$ by solving the following equations.

$$\begin{cases} \Sigma = \dfrac{k_p}{1 - \dfrac{\beta_2}{k_p}} \\ \tau_1 \coloneqq \dfrac{k_1}{b_1} = \dfrac{1}{2}\left(\dfrac{\mu + \Sigma \cdot \alpha_1}{\Sigma} + \sqrt{\left(\dfrac{\mu + \Sigma \cdot \alpha_1}{\Sigma}\right)^2 - 4\dfrac{\Sigma \cdot \alpha_0 + \nu}{\Sigma}}\right) \\ \tau_2 \coloneqq \dfrac{k_2}{b_2} = \dfrac{1}{2}\left(\dfrac{\mu + \Sigma \cdot \alpha_1}{\Sigma} - \sqrt{\left(\dfrac{\mu + \Sigma \cdot \alpha_1}{\Sigma}\right)^2 - 4\dfrac{\Sigma \cdot \alpha_0 + \nu}{\Sigma}}\right) \\ \mu \coloneqq \tau_1 k_1 + \tau_2 k_2 = -\dfrac{\Sigma^2}{{k_p}^2}(\beta_1 - \alpha_1\beta_2) \\ \nu \coloneqq \tau_1\tau_2(k_1 + k_2) = \dfrac{\Sigma^2}{{k_p}^2}(\beta_0 - \alpha_0\beta_2) \end{cases}$$

In this study, we performed the indentation with 4 types of cells and the system parameters, i.e. the viscosity and elasticity properties of cells, were identified using both the modified model and the original model. The viscosity and elasticity characteristic parameters were then averaged for each type of cells, as listed in Table 1. The comparison between the average parameters identified by the modified model and the original model shows that the viscoelastic parameters of the cells have changed to some extent by considering the effect of the AFM cantilever, indicating the necessity of consideration of the effect of the AFM cantilever.

IV. Conclusion

In this paper, the effect of the AFM cantilever was neglected and the viscoelastic properties of both the cell and the AFM cantilever are considered as a whole in the model. In this study, we modified the model by considering the AFM cantilever as an independent factor and the elasticity of AFM cantilever and the viscoelasticity of cells are decoupled. The modified model can characterize the mechanical properties of single cells without the influence of AFM cantilever. The system order and parameters were identified using the Hankel matrix method. The proposed model was validated by AFM indentation experiments with different types of cells. Four types of cells with twenty cells of each type were evaluated to collect the stress-relaxation data to validate the proposed dynamical model; the dynamical system for all the cells used in this study was determined to be second order. The spring constant of the AFM cantilever can be per-determined by thermal tune. Therefore, the cellular system model contained 3 elasticity parameters and 2 viscosity parameters, which represent the elasticity and viscosity characteristics, respectively, of cell dynamics. In other words, with the proposed model, the nonlinear elasticity and viscosity properties of a single cell can be decoupled, and the nonlinearity characteristics of each property can be described by multiple parameters in a linear system, and the viscoelastic parameters which were obtained by this improved model can eliminate the effect of cantilevers with different spring constant. Future work will be conducted to investigate the effects of drugs on the elasticity and viscosity parameters of cells using the proposed model.

Table 1. Parameters of four types of cell of average.

Cellular Types		Elastic Parameters (N/m)			Viscosity Parameters (N· s/m)	
		k_0	k_1	k_2	b_1	b_2
MCF-7	Modified	2.433±1.055	1.457±0.691	1.293±0.372	0.191±0.014	2.668±1.137
	Original	2.984±0.742	1.110±0.372	1.385±0.114	0.118±0.306	3.108±1.618
Neuro-2a	Modified	2.029±1.290	1.742±0.752	1.379±0.304	0.272±0.121	2.583±2.119
	Original	2.425±0.589	1.519±0.286	1.259±0.121	0.242±0.296	1.934±0.891
Hek-293	Modified	0.968±0.509	0.480±0.267	0.670±0.287	0.130±0.095	5.354±4.036
	Original	1.099±0.496	0.472±0.203	0.609±0.090	0.158±0.244	1.956±0.796
L-929	Modified	0.526±0.095	0.195±0.093	0.232±0.048	0.0200±0.009	0.323±0.150
	Original	0.534±0.096	0.195±0.067	0.235±0.009	0.0124±0.049	0.374±0.150

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