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Atomic force microscopy indentation and inverse analysis for non-linear viscoelastic identification of breast cancer cells

Q2 Nhung Nguyen[a,b], Yue Shao[a], Alan Wineman[a], Jianping Fu[a], Anthony Waas[b,*]

[a] Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI 48109, United States
[b] William E. Boeing Department of Aeronautics and Astronautics, Seattle, WA 98195-2400, United States

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ABSTRACT

Breast cancer cells (MCF-7 and MCF-10A) are studied through indentation with spherical borosilicate glass particles in atomic force microscopy (AFM) contact mode in fluid. Their mechanical properties are obtained by analyzing the recorded reaction force–time response. The analysis is based on comparing experimental data with predictions from finite element (FE) simulation. Here, FE modeling is employed to simulate the AFM indentation experiment which is neither a displacement nor a force controlled test. This approach is expected to overcome many underlying problems of the widely used models such as Hertz contact model due to its capability to capture the contact behaviors between the spherical indentor and the cell, account for cell geometry, and incorporate with large strain theory. In this work, a non-linear viscoelastic (NLV) model in which the viscoelastic part is described by Prony series terms is used for the constitutive model of the cells. The time-dependent material parameters are extracted through an inverse analysis with the use of a surrogate model based on a Kriging estimator. The purpose is to automatically extract the NLV properties of the cells with a more efficient process compared to the iterative inverse technique that has been mostly applied in the literature. The method also allows the use of FE modeling in the analysis of a large amount of experimental data. The NLV parameters are compared between MCF-7 and MCF-10A and MCF-10A treated and untreated with the drug Cytochalasin D to examine the possibility of using relaxation properties as biomarkers for distinguishing these types of breast cancer cells. The comparisons indicate that malignant cells (MCF-7) are softer and exhibit more relaxation than benign cells (MCF-10A). Disrupting the cytoskeleton using the drug Cytochalasin D also results in a larger amount of relaxation in the cell's response. In addition, relaxation properties indicate larger differences as compared to the elastic moduli like instantaneous shear modulus. These results may be useful for disease diagnosing purposes.

1. Introduction

The knowledge of mechanical characteristics of biological cells is essential for various applications. Such an application is the manufacture of engineered polymeric synthetic cells that can mimic and replace real cells [1]. For this purpose, it is necessary to make synthetic cells mechanically resemble real cells by matching their mechanical properties. Therefore, understanding cells' mechanical responses is beneficial in the selection of materials for the manufacture process as well as examining the effectiveness of the engineered cells. Another application is related to the study of the physiology of biological cells. As many cells' functions and processes have been known to be significantly influenced by external mechanical stimuli [2–7], the knowledge of cell mechanics might lead to better quantification of many cells' physiological mechanisms. Additionally, cell mechanics is closely linked with alterations in cytoskeletal structures, which may be associated with invasive diseases such as cancer. Therefore, such knowledge might also play an important role in formulating potential biomarkers for disease detection. For example, the difference in cell stiffness between cancerous and their corresponding normal cells have been investigated in several studies [8,9]. Initial results indicated that certain types of diseased cells might be softer and more deformable. Furthermore, changes in cell stiffness have been reported between different states of cancer. The studies in [10–13] showed that as cells transform from benign to malignant stages, the stiffness exhibits a decreasing trend. In addition to stiffness, the viscoelastic behavior of the cells has also been observed [12–15], but this still requires further investigation, especially at the large deformation range. Preliminary results suggest

Q4 * Corresponding author. Tel.: +1 2066171362.
E-mail address: awaas@aa.washington.edu, dcw@umich.edu (A. Waas).

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that viscoelastic characteristics may be effective indicators and biomarkers for better diagnosis and treatment of this dangerous disease, and could also aid testing the efficiency of anti-cancer drugs to combat cancer.

Currently, in order to obtain the mechanical characteristics of biological cells, techniques such as micropipette aspiration [2,16–18], atomic force microscopy [2,6], magnetic twisting cytometry [2,3,19], and optical trap [20] based testing are available. Amongst them it appears that contact of a cell with a "rigid" indentor, followed by measurement of the sample response to controlled motion of the indentor, is a common technique by which the cell properties are probed. The measured data is then interpreted by formulating and solving a corresponding boundary value problem with an assumed constitutive model for the cell material. However, this interpretation is still a challenging problem and highly dependent on the chosen model. In the case of AFM indentation test [6,11–13,21,22], the Hertz contact model [23] has been frequently used to analyze the data. Nevertheless, this model is only valid under the restrictions of many assumptions such as small strain, infinitesimal elastic deformation [23,24] which are not normally satisfied in the actual context of an AFM indentation test. In order to account for the cases where large deformation occurs, AFM indentation tests should be studied using approaches which can include the consideration of non-linear mechanics. Such an approach interprets the experimental data using the FE method to take into account many aspects of the indentation process, including the dimensions of the probe and the sample, contact features, and non-linear material models [24,25]. This method can better capture the response at the large strain range but the computational cost associated with FE models is a drawback for inversely extracting material properties [26]. Furthermore, the number of unknown material parameters needed to identify mechanical behavior and the large amount of experimental data increase the difficulty of the inverse process. In addition, in determining the nonlinear viscoelasticity of biological cells, a complex platform of experimental inputs that is composed of different loading histories might be needed [24,27], contributing to an increased cost in the inverse analysis. In the literature, recent work by [24] employed an inverse FE approach for interpreting AFM experimental data. Nevertheless, details related to these computational issues have still not been thoroughly discussed. Additionally, in many cases of AFM indentation, the experiment is neither force nor displacement controlled because of the manner by which an AFM operates. This is normally neglected during FE modeling, which is also another source of inaccuracy.

This paper focuses on using AFM indentation techniques to investigate the correlation between NLV properties of breast cancer cells (MCF-7 and MCF-10A) and alterations in the cytoskeletal structures utilizing two approaches. In the first, breast cancer cells at the benign (MCF-10A) and malignant (MCF-7) states were indented in their culture medium using spherical probes in AFM contact mode in fluid. A two-step indentation loading input was employed. It was comprised of applying a small force to initiate the contact between the probe and the cell, followed by controlling the AFM piezo movement in a ramp-reverse and ramp-hold manner. In the second, the same indentation procedure was applied to investigate the effect of the drug induced cytoskeletal structure on the cells' NLV characteristics due to a drug treatment with Cytochalasin D. The indentation experiment was simulated using the FE method in which the cell material constitutive relationship is a large strain viscoelastic model. The non-linear elastic part of the material is captured by a hyper-elastic model while the viscoelastic part composes of a two term Prony series to describe the time dependent relaxation. In order to simulate the actual operation in the AFM, the FE model also includes the AFM cantilever in the modeling by using a spring with the same stiffness. Once an FE model is constructed, an inverse analysis is needed to optimize the error between the experimental data and FE predictions. The iterative inverse approach is computationally expensive, as noted in [26]. Therefore an inverse technique based on a surrogate model with the use of a Kriging estimator [27–33] is employed to address this issue. The procedure allows an automatic and efficient extraction of the NLV parameters. The variation of the shear relaxation modulus for each tested cell is, therefore, obtained. Statistical comparisons using the shear relaxation modulus and the amount of relaxation for the two cases (MCF-7 versus MCF-10A, and untreated versus treated MCF-10A) were also conducted to investigate differences in terms of these mechanical properties.

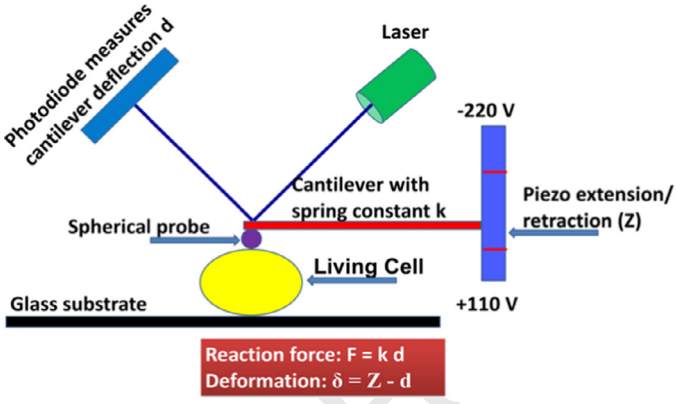

Fig. 1. Illustration of working principle of AFM indentation test on cells.

2. Experiment details

2.1. Sample preparation

Human mammary epithelial cells (MCF-10A) were cultured in mammary epithelial growth medium (MEGM, Lonza) with the GA-1000 replaced by 100 ng/ml cholera toxin (Sigma). Human breast cancer cell line (MCF-7) was maintained in Dulbecco's modified eagle medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 1% penicillin/streptomycin (P/S, Life Technologies), 1% fungizone (Life Technologies), and 5 g/ml gentamycin (Life Technologies). For preparing samples for AFM tests, cells were resuspended and seeded onto glass coverslips at density of 40,000–80,000 cells per coverslip. Cells were cultured under 37 °C and 5% CO_2 for at least overnight before any test was performed. For pharmacological treatment assays, MCF-10A cells were incubated with 500 μM Cytochalasin D (Life Technologies) for 2 h and then tested by indentation.

2.2. AFM indentation experiment setup

The working principle of an AFM Bruker Dimension Icon instrument [34] is illustrated in Fig. 1. The sample is fixed to a rigid substrate and compressed by a probe attached to one end of an AFM cantilever (for indenting cells, a spherical probe is often used to reduce damage during the tests). The other end of the cantilever is connected to a piezo. As this piezo extends, the probe moves downward and comes into contact with the sample. On the other hand, as the piezo retracts, the probe moves upward.

Upon contact, the sample's reaction force F causes the cantilever to bend leading to a shift of the laser beam on the photodiode. The amount of shifting is related to the deflection d of the cantilever, which is in turn related to the reaction force by a linear relation $F = kd$. Here, k is the spring constant of the cantilever calibrated using the built-in thermal tune function in the AFM instrument [35]. The deformation δ of the sample is the difference between the piezo distance Z and the cantilever deflection

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