Ultra-compliant Thermal AFM Probes for Studying of Cellular Properties

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***Abstract*- Atomic force microscopy (AFM) can be used for a number of nanoscale biological studies. It opens the possibility to monitor cellular processes in physiological conditions with the ability to perform high resolution imaging and force measurements. However, analysis of the mechanical property of the living cells is difficult and the sensitivity is low. Conventional AFM probes use high-stiffness materials and therefore, it makes the force measurement on soft samples becoming more difficult. In this work an ultra-compliant AFM probe made of polyimide was used to provide an improved imaging and force measurement of cancer cells. The probe includes an embedded sensing element for thermal conductance characterization. The probe was used for thermal conductance and topographical mapping of biological cells. The probe allows for high speed imaging of cells in liquids.**

I. INTRODUCTION

Single cell disease markers offer an inexpensive way to quickly test possible drug candidates on live cells before testing the drugs on animals or humans. At the same time, new personalized treatments tested on single live cells, rather than one-size-fits-all treatments like chemotherapy, have an excellent likelihood of effectively treating diseases in the future. The B cells lymphoma is cancerous form of B cells found in non-Hodgkin lymphoma. Rituximab (commercially named Rituxan or MabThera) is a chimeric monoclonal antibody against the protein CD20, which is primarily expressed on the surface of B cells. By a series of cascade effects, it induces the apoptosis (programmed cell death) of the CD20 positive B cells lymphoma [1]. The elimination of the cancerous B cells will eventually allow the development of healthy B cell population from the lymphoid stem cells. Clinically it is mainly used for the treatment of many lymphomas and leukemias. It is well established that Rituximab therapy alone results in a clinical response rate of 50%, yet it is unclear why the remaining 50% of patients do not respond [2]. Besides, a majority of responsive patients acquire resistance to further Rituximab therapy [3]. AFM can be used to find the possible biomarkers of cell properties. Recent research on cancer cells also showed that the adhesion brushes on the cell membrane can be used for the detection of cancer in single cells [4][5].

In recent years, AFM is increasingly being used for biological and medical applications [6]-[8]. It has proved to be an effective tool for quantifying the elasticity of various types of cells, such as metastatic lung-, breast, and pancreatic cancer cells [9], as well as benign and cancerous human breast epithelial cells [10]. Using AFM, metastatic cancer cells have been shown to exhibit much lower elastic stiffness than their benign counterparts, likely reflective of cytoskeleton changes associated with cellular transformation [9]. Additionally, AFM indentation studies have shown that age-related morpholical and biochemical changes in articular cartilage can be detected by force measurements [10]. These studies indicate that AFM has the potential to provide a link between the structural and mechanical properties of cells and their cellular function in the context of physiological and disease states.

Conventional AFM cantilevers for force measurements typically use silicon nitride or silicon as structural materials. However, the mechanical property measurement on soft samples such as cells is suffered by the high stiffness of the AFM probe.

In this work, ultra-compliant polyimide-based AFM probes were fabricated and used to conduct a number of measurements on B cells. Thermal images of B cells lymphoma were performed. The lower Young's modulus of polyimide allows for improved imaging of soft samples. In addition, force-displacement curve measurements in liquid on the cells were conducted in order to study the mechanical properties of the B cells lymphoma. These probes measure cell stiffness with higher accuracy. Finally, high speed imaging of cells in liquid was demonstrated. These results demonstrate that polymer-based probes are well suited for liquid measurements of biological cells opening new possibilities for AFMs in cell biomarkers.

II. MATERIALS AND METHODS

A. Design and Fabrication of Thermal AFM Probes

The structure of the polyimide probes is shown in Fig. 1. The probe tip has a diameter of 100 nm, which can be reduced about 50 nm with oxide sharpening. It offers topographical resolution of <1 nm and a spring constant of 0.141 N/m. The tip height is 8 μm, and the cantilever is 350 μm long, 90 μm wide, and 3 μm thick.

The probes are batch fabricated in a process described in [11]. The probes were micro-fabricated in a seven masking step sequence. Initially, a mold for the tip was created by anistropic wet etching on a (100) Si substrate. Then a

sacrificial layer was deposited and patterned, followed by the lower polyimide and the metals. Later, the second polyimide layer was deposited and patterned, followed by a gold layer, which was used for thermocompression bonding and served as a mirror. Finally, the probe was released, flipped over, and held in place by a thermocompression bond.

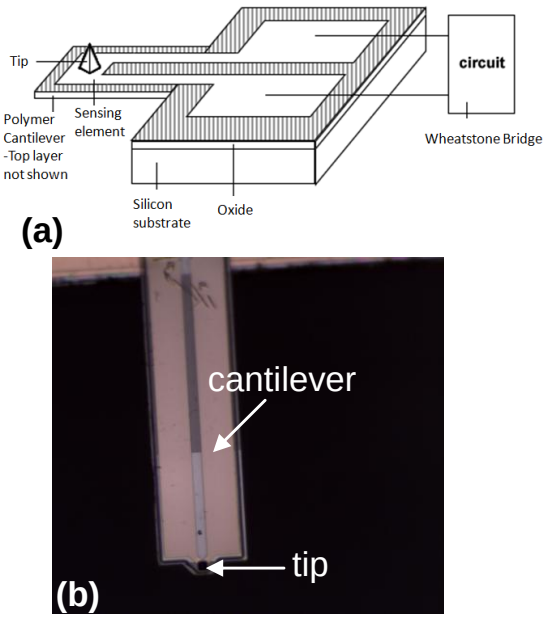

Fig. 1. (a) Schematic drawing of the polyimide probe including the probe cantilever and tip [6]. (b) Optical image of a probe.

B. Cell Culture and Sample Preparation

B cells lymphoma cells (ATCC.org) were grown to confluence in RPMI-1640 medium (Gibco-Invitrogen, Carlsbad, CA USA.) supplemented with 10% fetal calf serum (Gemini Bio-products, West Sacramento, CA USA.) and 1% penicillin at 37°C in a humidified atmosphere containing 5% CO2. The cells were then plated onto poly-L-lysine (Sigma, St. Louis, MO USA) coated glass cover slips for initial attachment. They were then fixed using 3.7% faraformaldehyde (Invitrogen, Carlsbad, CA USA). Glass cover slips were transferred to the AFM for imaging.

C. AFM Imaging

We used a Bioscope AFM (Veeco Instruments, Woodbury, NY) for imaging living cells, which is equipped with a scanner with a maximum XY scan range of 90μm x 90μm and a Z range of 5μm. Peripheral devices including an optical microscope and a charge-coupled device (CCD) camera are also connected to the AFM. AFM imaging was conducted in air on control B cells and CD20 antibody treated B cells. The CD20 antibody for the B cell treatment was used to induce cell apoptosis, and therefore change cellular properties. All scans were completed by taking 256 × 256 point scans and recording topographic data. Both the trace and retrace images were measured and compared.

The polyimide probe was operated with an AFM. The thermal information from the probes was fed to a circuit module, which in return interfaced with an AFM controller. The probe was operated in a contact mode by scanning a thermal probe tip across a sample and making measurements at discrete points. Thermal probes operated in contact mode show improved performance. While operation in a tapping or non-contact mode has several disadvantages. First, the temperature sensitivity of the probe is compromised because of the large thermal resistance of the air gap. Second, spatial resolution is reduced because the effective sensing area is enlarged as the distance between the sensor and the sample increases. Third, high stiffness in the probe is required which may cause damage to soft samples. The use of polyimide probes eliminates these problems.

III. Experimental Results

A. AFM and Thermal Imaging

Contact mode AFM topographical images of non-treated and antibody treated cells are shown in Fig. 2a and Fig. 2c respectively. Thermal images (Fig. 2b and Fig. 2d) of the non-treated and antibody treated samples were obtained simultaneously. A good correlation between the topographical images and the thermal images can be observed. By comparing the thermal images before and after antibody treatment, we can observe that the antibody treated B cell has lower thermal conductance than the control sample, this result could be used as a potential biomarker for cell studies.

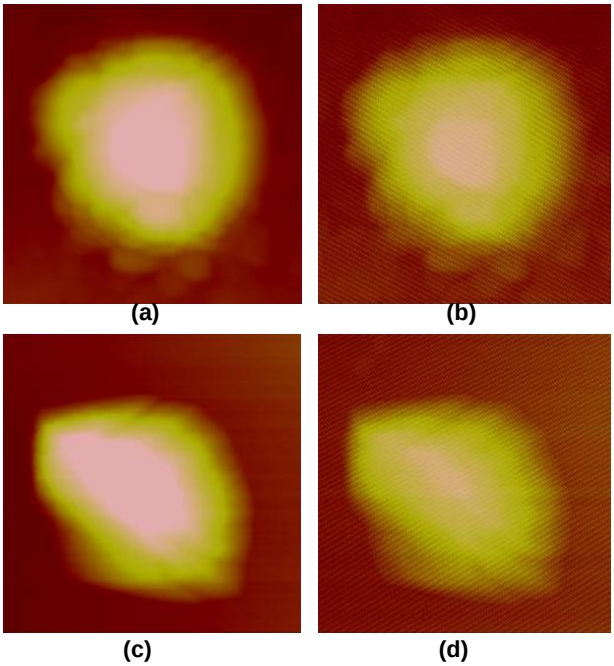

Fig. 2. (a) Height image and (b) thermal image of the B cells lymphoma without antibody treatment (scan size: 8.1 μm). (c) Height image and (d) thermal image of the B cells lymphoma with antibody treatment (scan size: 6.7 μm).

B. Force Measurements

Force-displacement curves on a glass slide showed that these probes have a deflection sensitivity of approximately 200 nm/V (compared to silicon nitride probes that have <100nm/V). The spring constant of these probes is 0.141 N/m. These two properties allow for a greater sensitivity when measuring the force-displacement curve. These two properties also allow for the detection of small changes in the adhesion forces during the force-displacement curve measurements. The adhesion force is the snap-off part of the force displacement curve.

Fig. 3a and

Fig. 3b show the difference in the adhesion force between the control sample and the antibody treated sample. This measurement was conducted in liquid. Fig. 3 implies that the adhesion force can also be used as a cellular biomarker. Fig. 4 shows the Young's modulus values at different locations of a cell derived from force-displacement curves. The cell was in liquid.

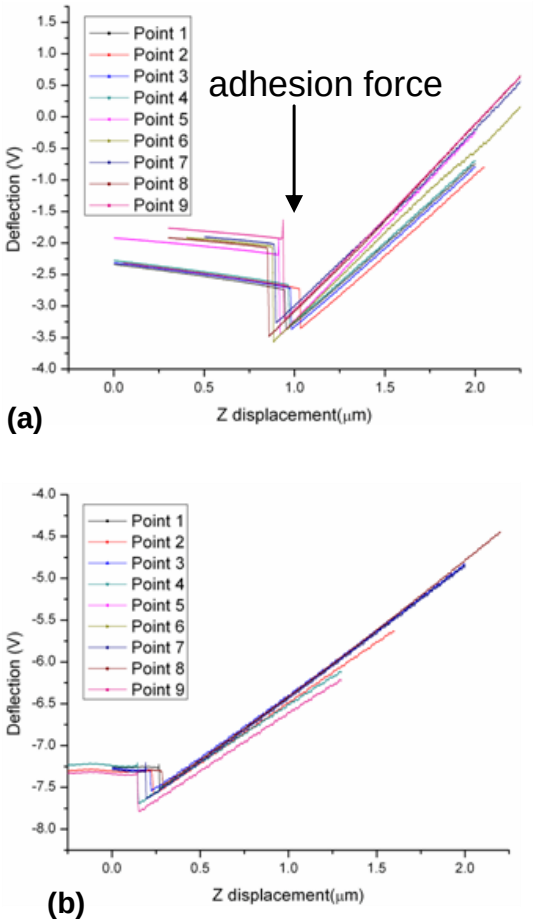

Fig. 3. Force-displacement curves of the B cells lymphonma (a) without antibody treatment and (b) with antibody treatment.

The force curves, which show the deflection of the cantilever as the function of the movement of the piezoelectric scanner in Z (vertical) direction, can be converted to force indentation curves which can be further analyzed and fitted with the classic Hertz continuum mechanics model in order to quantify the elasticity of the cell.

For a paraboloid indenter, the Hertzian model can be expressed as [12]:

$$F = \frac{4}{3}\frac{E}{1-\nu^2} R^{\frac{1}{2}} \delta^{\frac{3}{2}} \qquad (1)$$

where $\boldsymbol{E}$ is the Young's modulus, $\boldsymbol{\nu}$ is the Poisson's ratio and $\boldsymbol{R}$ is the radius of curvature of a paraboloid indenter. $\boldsymbol{\delta}$ is the indentation and $\boldsymbol{F}$ is the indentation force.

Therefore the fitting parameter $\boldsymbol{P}$ would be:

$$P = \frac{4}{3}\frac{E}{1-\nu^2} R^{\frac{1}{2}} \qquad (2)$$

After finding the fitting curve for the force-indentation curve of living cells, Young's moduli at four different points (*P1, P2, P3 and P4*) were obtained as shown in Fig. 4b. Young's moduli at points *P1* and *P4* are higher because of higher stiffness. This is due to the edge effect of the substrate.

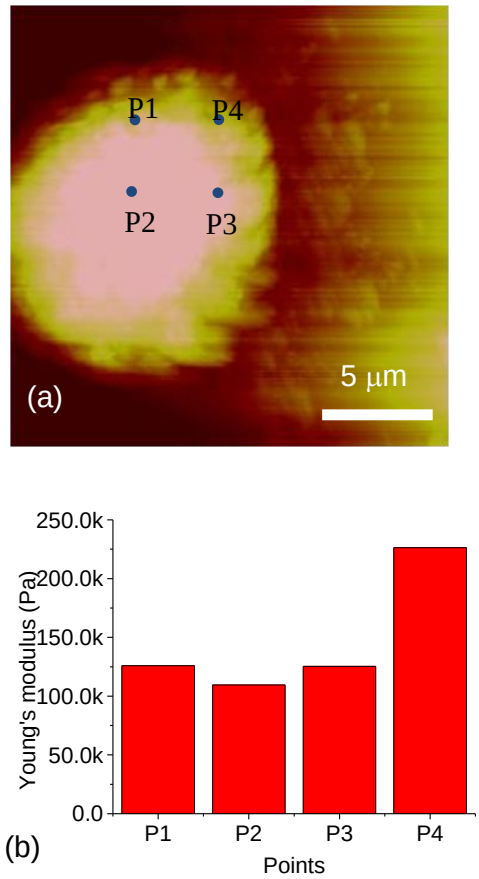

Fig. 4. (a) Atomic force microscopic image of fixed B cell image in PBS by polymer probe. (b) Young's modulus calculated from force-displacement curves taken on different locations of the fixed B cell (P1, P2, P3 and P4).

C. *High Speed Imaging*

In order to monitor cellular processes over time, the time for the AFM imaging and force measurement is critical because the cells need to maintain in physiological conditions. Scan speeds of current tapping mode AFMs are limited to about 180-250 μm/sec due to actuation time constant of the piezo-electrically actuated feedback loop [13][14]. A typical value for contact mode imaging is about 75 μm/sec [15]. Since the thermal probe is softer than a normal silicon probe, the scan speed of the current AFM by using the thermal probe can be further increased. High-speed imaging of the B cell in liquid was performed and AFM topographical images were obtained as shown Fig. 5.

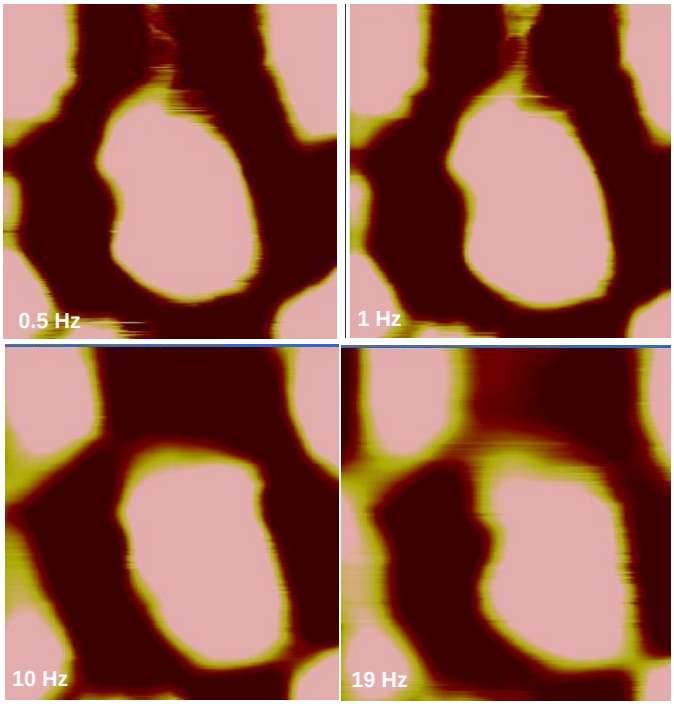

Fig. 5. Atomic force microscopic images of fixed B cell in PBS were obtained under different scan rate. The scan size of the images are 25 μm x 25 μm.

IV. Conclusion

To conclude, an ultra-compliant surface micromachined scanning thermal probe has been developed and used to study the thermal and elastic properties of live and fixed cells in and out of liquid. The probe uses polyimide as the structural material and an embedded thin film metal resistor as the sensing element. The advantages of using the thermal probe for cell studies have been discussed. We demonstrate that the polyimide probe can be operated with AFM and used for measurement the mechanical properties of B cells lymphoma before and after antibody treatment. In addition, the polyimide probe offers the ability to perform the high-speed imaging, which is important for living cells studies in physiological conditions.

Acknowledgment

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