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Development and Testing of Nano Robot End Effector for Cell Electrophysiology and Elastography Studies

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***Abstract* — Electrophysiology and elastrography provide significant information in biological and biomedical studies. Conventional atomic force microscopes (AFMs) have been used for the mechanical characterization of living cells, but they lack the capability to measure the electrical properties of biological cells and tissues. In this work, a novel nano robot end effector has been developed to perform multidimensional measurement of cells. The end effector is designed for characterization of soft materials and it includes an embedded metal electrode for membrane potential measurement of cells.**

Index Terms - atomic force microscopy, electrophysiology, elastography, single cell analysis.

I. INTRODUCTION

Development of new microscopic and measurement techniques is becoming a driving force in the advancement of life sciences and the pharmaceutical industry. The physiological observation of single live cells provides new information and disease biomarkers [1][2].

Atomic force microscopy (AFM) is an effective tool for biological studies [3][4]. It has been used for quantitative studies of mechanical properties of various cells type such as pancreatic cancer cells [5] and cancerous human breast epithelial cells [6]. Using an AFM, metastatic cancer cells have been shown to exhibit much lower elastic stiffness than their benign counterparts, which is probably reflective of cytoskeleton changes associated with cellular transformation [5]. These studies indicate that AFM has the potential to provide a connection between the structural and mechanical properties of cells. Currently, AFM systems are limited to obtaining the mechanical properties of cells. It is speculated that the mechanical structure of a cell correlates to its ion channel activities [7]. Patch clamps are employed to measure membrane potentials, which relate to ion channels activities [8]. Defects in ion channel function point to various diseases, and as a result ion channels are important targets for drugs. Adding electrophysiology capability to AFM and generally to scanning probe microscopes opens the possibility to: create links between mechanical and electrical properties, discriminate between healthy and disease states, and identify various cellular functions of cells.

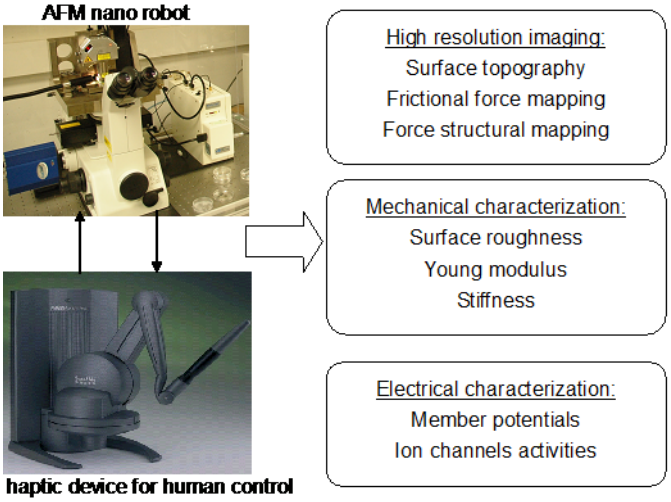

Fig. 1. Illustration of the AFM-based nano robot for multidimensional characterization of biological cells.

In this paper a polymer based nano robot end effector is introduced. The end effector has been integrated with an AFM-based nano robot enabling simultaneous high-resolution mechanical and electrical characterization of live cells. The system can also be used for the study of DNA or other biomolecules [9]. In addition, the system allows for nanomanipulation of single cells, feature detection, and morphology imaging. Elastography is obtained by force-displacement measurements. Membrane potentials can be measured with an electrode on the end effector indicating the

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ion channel activity of a cell. New cellular biomarkers can be developed by combining elastography and electrophysiology measurements.

II. Design of the Nano Robot End Effectors

Nano robot end effectors like AFM probes are typically made from silicon or silicon nitride. Here, a novel end effector made from polyimide has been developed as shown in Fig. 2 and Fig. 4. Polyimide offers higher compliance and robustness. The end effector includes a tip with a diameter of < 100 nm and height of 8 μm. The end effector also includes an electrode, which is sandwiched between two layers of polyimide and covers the tip. The end effector offers a topographical resolution of < 1 nm and a spring constant of 0.141 N/m. The cantilever is 150 μm long, 100 μm wide, and 2 μm thick. The tip is insulated except for a tiny hole at the tip apex designed to provide electrical contact to the cell membrane for electrophysiology measurement.

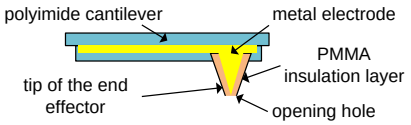

Fig. 2. (a) Illustration of the polyimide-based end effector including the soft cantilever and opening hole for electrical measurements.

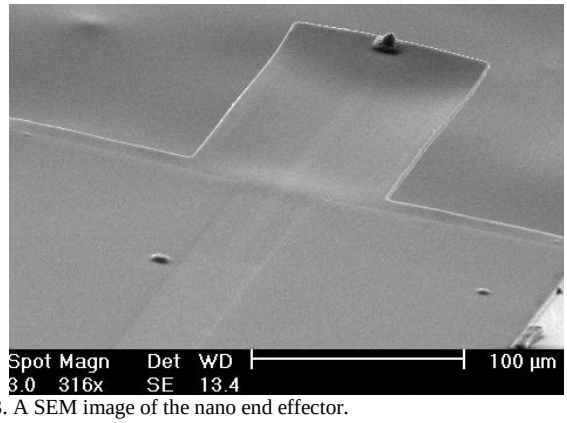

Fig. 3. A SEM image of the nano end effector.

A new surface micromachining process was developed to fabricate the end effectors. The process is described in Fig. 4. First, the tip of the end effectors is made on an SOI wafer by KOH etching. Then the first layer of polyimide is deposited and patterned, followed by metal (Ti/Pt) deposition and patterning. The second polyimide layer is deposited and patterned. The SOI substrate is back etched by deep reactive-ion etching (DRIE) to release the cantilever. A layer of aluminum is evaporated on the backside of the cantilever for AFM reflection. Finally, in order to create a nanoscale opening just at the tip while insulating the rest of the tip and cantilever, PMMA was deposited and using electron beam lithography and a nanoscale opening was created at the apex of the tip.

The nano end effector was operated with an AFM in contact mode. A Bioscope AFM (Bruker Nano, Santa Barbara, CA, USA) for live cell imaging, electrophysiology, and elastography measurements was used. The system is equipped with a piezoelectric scanner with an XYZ range of 90 μm × 90 μm × 5 μm. The system includes an optical microscope with a CCD camera for macro-scale observation. AFM imaging was conducted by obtaining 256 × 256 point scans and recording topographic data. The AFM system has been modified to perform nanomanipulation with an augmented reality interface with real-time visual display and force feedback during nanomanipulation [10][11]. The augmented reality interface lets the user guide the end effector to target locations much like a joystick.

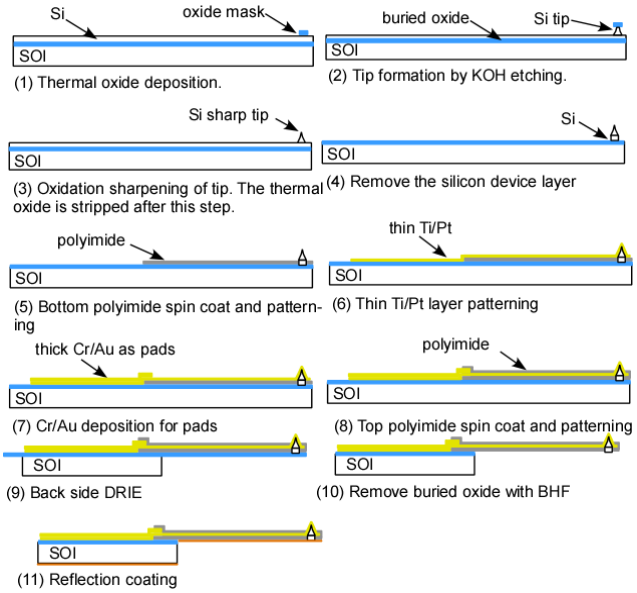

Fig. 4. Fabrication processes of the end effector.

III. Electrophysiology Measurement on Pancreatic Beta-Cells

Pancreatic β-cells are one of the cells in the endocrine pancreas that makes insulin. Insulin is a hormone involved in blood glucose homeostasis by ion channels regulation. In stimulus-secretion coupling process, β-cells trigger their insulin secretion in response to the stimulation of glucose, so the β-cells are the major physiological units in the pancreas. Recently, we used an AFM to study the mechanical properties of pancreatic β-cells [13]. The electrophysiological properties and the function of ion channels of β-cells are extremely important. Defect in ion channels would cause severe metabolic dysfunction resulting in diseases such as diabetes. Thus the investigation of β-cell ion channel activities enhances our understanding of the stimulus-secretion coupling and facilitates the diagnosis and treatment of related disease. Electrophysiological patch-clamping, "whole-cell recoding" [14], were conducted on pancreatic β-cells using the end effector in the experimental setup shown in Fig. 5. The AFM end effector tip ruptures the cell membrane, so that tip's metal electrode is in contact with the cell's cytoplasm. As a result the electrode has the same potential as the membrane.

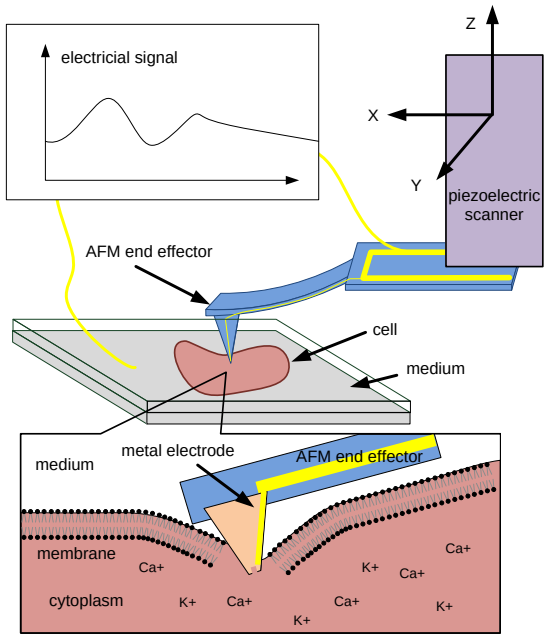

Fig. 5. Illustration of the electrophysiology measurement with the nano robot end effector.

Before the experiment, the insulinoma cells (ATCC, Manassas, VA) were cultured to confluence in RPMI-1640 medium (Gibco-Invitrogen, Carlsbad, CA USA) supplemented with 10% fetal calf serum and 1% penicillin at 37 °C in a humidified atmosphere containing 5% CO_2. They were then seeded onto glass coverslips until reaching confluency. Before each glucose stimulation experiment, the cells were placed in a petri dish with 5 ml of low glucose medium (2 mM) for around 90 minutes until reaching equilibrium. For glucose level stimulation, 10 µL of high glucose medium (2 M) would be added to the petri dish during the measurement. The experiment was performed using a voltage clamp, i.e. the membrane potential was applied by the end effector and the current was measured. The current recording data were obtained and presented in time-course as shown in Fig. 6.

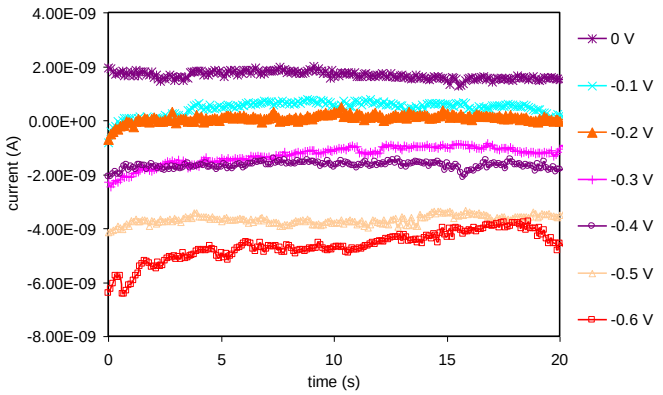

Fig. 6. The whole-cell current data recorded for the pancreatic β-cell at different potentials.

In the experiment, the membrane potential was held at 0 V initially, and then the voltage was reduced to increasingly lower negative potentials. As a result, the current shifted from positive values to negatives values. The same current data were plotted in the form of a current-voltage curve ranging from -0.6 V to 0 V shown in Fig. 7. These results demonstrate electrophysiology measurements using the nano end effector.

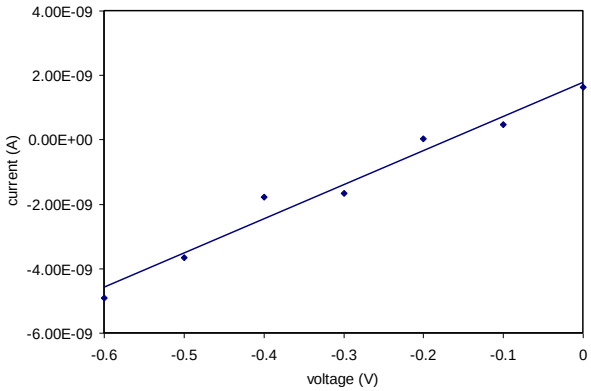

Fig. 7. Current-voltage relationship (I-V) of the pancreatic β-cell after glucose stimulation.

IV. Elastography Measurement on B Cells Lymphoma

B cells lymphoma are a cancerous form of B cells found in non-Hodgkin lymphoma. Rituximab therapy has been used to eliminate the cancerous B cells, increasing the population of healthy B cells. However, this therapy has a low positive response rate [15]. Single cell disease markers are needed to test possible drug candidates on live cells. AFM force-displacement elastography measurements (force-curves) may be used as biomarkers [16].

B cells lymphoma (American Type Culture Collection, Manassas, VA USA) 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 elastography measurement.

An elastography measurement of a B cell lymphoma is obtained by placing the end effector on a desired location of a cell and then moving vertically the end effector toward and away from the cell using the piezoelectric scanner, shown in Fig. 9. A laser aligned to reflect from the backside of the end effector tracks the deflection of the end effector by measuring the reflected signal using a photodiode. The force-curve slope, recorded as soon as the end effector contacts the cell membrane, can be used to derive the cell's stiffness/elasticity.

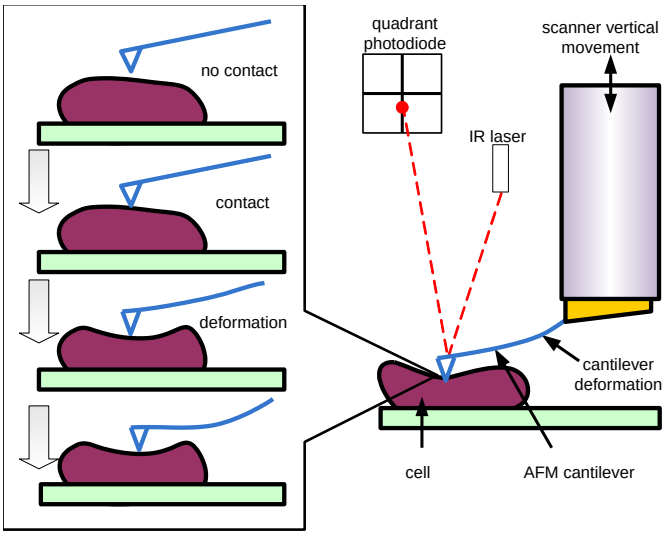

Fig. 8. Illustration of the deflection-displacement measurement (elastography measurement) of the B cells lymphomas.

In this experiment a target cell is identified by AFM imaging and then elastography measurements are conducted. An AFM image and deflection-displacement curves (force-curves) on the B cells lymphoma with antibody treatment are shown in Fig. 9a and Fig. 9b, respectively. The end effector has a deflection sensitivity of approximately 200 nm/V enabling high resolution elastography measurements of biological cells.

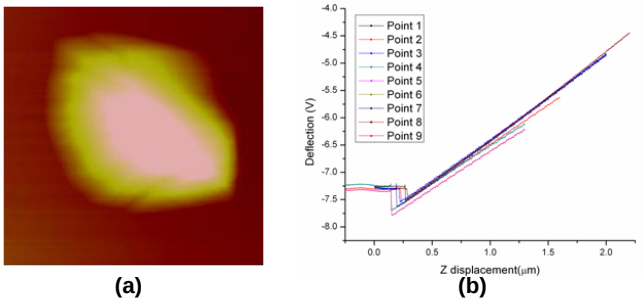

Fig. 9. (a) An AFM image of a B cell lymphoma with antibody treatment. (b) Deflection-displacement curves (force-curves) of a B cell lymphoma at various points on the cell.

V. Conclusion

Surface micromachined end effectors have been developed and tested. The end effectors use polyimide as a structural material for enhanced compliance and robustness. A metal electrode is embedded for patch clamping. The utility of the end effector has been demonstrated by conducting electrophysiology and elastography measurements on pancreatic β-cells and B cells lymphoma, respectively. These results demonstrated that the AFM end effectors are well suited for liquid measurements of live cells. Furthermore, this novel device may enable the development of new single cell disease biomarkers that combine electrophysiology and elastography measurements.

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