A PRACTICAL SINGLE CELL ANALYSIS METHOD FOR MECHANICAL CHARACTERIZATION OF CANCER CELLS

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ABSTRACT

This study presents a practical method to perform mechanical characterization of cells using Silicon Nano Tweezers (SNT). Integrated with a microfluidic device, SNT tips were inserted in a channel via a side opening. Cells to be characterized were injected to an inlet of the microfluidic channel and withdrawn by a vacuum pump connected to an outlet. Mechanical properties of a cell, e.g. diameter, stiffness and viscous losses, were analyzed after capturing between the SNT tips (a sensing tip and a compressing tip). After characterization, the captured cell was released to capture another cell. Repeated capturing and characterization steps allow us to test several cells and distinguish fixed and live SUM-159-PT cancer cells based on their mechanical properties.

INTRODUCTION

Breast cancer is the second leading cause of cancer death in women because of metastasis [1]. Circulating Tumor Cells (CTCs), shed from a primary tumor, are known as seeds for metastases. As the number of CTCs in blood determines the survival rate of metastatic patients, early CTC detection is a matter of life and death. The main challenge is that CTCs are extremely scarce in the blood (few CTC among few billion red blood cells in 1ml blood). Moreover, there is no single biomarker for all CTCs. Therefore, reliable, practical, time and cost efficient methods are in great need for early detection of CTCs.

Immunoaffinity-based microfluidic devices have been used to isolate CTCs in multi-method integration [2]. There are also other systems targeting the number of CTCs in a blood sample, e.g. CellSearch [3]. As immunoaffinity-based approach is costly and time-consuming, these methods are difficult to use as practical early detection systems. Moreover, cells going through some transitions are difficult to be detected by immunoaffinity resulting in a high risk of missing potentially important set of cells. Consequently, a non-labeled system targeting other properties, e.g. mechanical and/or electrical, can be a good candidate for early CTC detection as invasive cancer cell lines exhibit biomechanical properties that are distinct from their noninvasive counterparts [4].

Different techniques characterize CTCs based on their electrical or mechanical properties such as micro electrical impedance spectroscopy (MEIS)[5], real-time deformability cytometry (RT-DC)[6] and Atomic Force Microscopy (AFM) [7]. These techniques provide either limited information of a characterized cell (MEIS and RT-DC) or suffer from low-throughput refraining them from practical use (AFM). CTC detection and characterization benefit from higher information content (for practical methods) or higher throughput (for AFM).

MEMS technology allows performing assays on mechanical responses of macromolecules in a rapid, efficient, reliable and cost-effective way. We have recently demonstrated MEMS tweezers (with integrated actuator and sensor) monitoring bio/chemical interaction of biomolecules e.g. DNA bundles, in real-time [8]. Using an appropriate microfluidic design, tweezers can perform bioassays without compromising stability and sensitivity. Easy setup and automation possibility with supreme handling capabilities of MEMS technology provide the required higher throughput without compromising the information content.

Here, we present a similar method modified for cell characterization. Flat tips at the end of two parallel arms were designed to manipulate single cells. One of the arms, the compressing arm, compressed a cell (using integrated comb-drive actuator) while the opposing one, the sensing arm, performed harmonic detection (using integrated displacement sensor; Fig. 1a). The tips were inserted in a microchannel via a specifically designed side opening (Fig. 1b). Cells were inserted in the channel inlet and transported towards the tips using a pump connected to the outlet. Automated insertion and detection system allowed rapid, cheap and sensitive mechanical characterization of cells.

MATERIALS AND METHODS

Silicon NanoTweezers

SNT had two mobile tips actuated by separate comb-drive actuators and a differential capacitive sensor integrated with one of the arms, i.e. the sensing arm (Figure

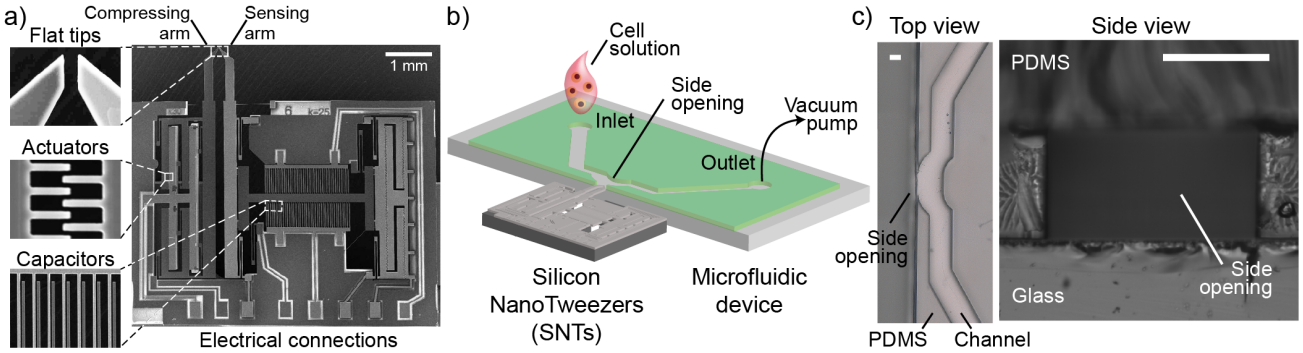

Figure 1: ***:*** *a) SNTs had compressing and sensing arms connected to a displacement sensor (capacitors) and comb-drive actuators. b) Tips were inserted in a microfluidic channel via a side opening. Connecting the outlet to a vacuum pump, a cell solution were injected and different cells were brought to SNT tips for analysis. c) The side opening at the edge of a cover slip provided only the SNT tips to enter without compromising stability. Scale bars correspond to 100µm.*

1a). The sensing arm could perform characterization while the opposing arm, the compressing arm, were capturing and squeezing a cell (uniaxial compression). The sensing arm actuated with a resolution of 0.2nm and a maximum displacement of 2µm by a comb-drive actuator to perform harmonic measurements. The compressing arm, on the other hand, required a larger stroke to compress cells. The other comb-drive actuator provided a maximum displacement of about 10µm (at a potential difference of 100V). As a result, tips with a gap of 18µm could capture a cell of 16µm diameter and compress up to 50% of the initial diameter.

The measurements were performed using harmonic oscillation of the sensing arm ($1V_{p\text{-}p}$ actuation). The mechanical resonance of the system was monitored in real-time. A LabVIEW program controlled all devices to automate SNT positioning and cell compression while recording environmental and actuation sensor readings [8].

Microfluidic device

We fabricated a PDMS microfluidic device (by molding on SU8 structures) and placed on a cover slip (Fig. 1c). The device had an inlet, an outlet and a side opening (200µm x 110µm). A vacuum pump was connected to the outlet to withdraw the sample solution inserted via the inlet. The side opening was designed for SNT tips to access the channel. Therefore, a cell solution dropped on the inlet could be withdrawn by the vacuum pump to bring cells in the close vicinity of SNT tips at the side opening. The size of the side opening was small enough to keep the air-liquid interface stable due to the high surface tension but still large enough to allow SNT tips to maneuver for cell capturing.

Microfluidic device was positioned on a motorized stage. The piezoelectric elements of the stage allowed motion with 3 degree of freedom. Nanometric accuracy of this 3D robot provided high precision positioning of the microfluidic device with respect to the SNT tips. The robot was connected to a PC and controlled by LabVIEW program to automate positioning.

Preparation of cells

Live and fixed (by 5% PAraFormaldehyde) SUM-159PT breast cancer cells were used to demonstrate the proposed method. Fixation procedure disabled intrinsic biomolecules, protected the sample from extrinsic damage and altered cells at a molecular level to increase mechanical stability. Due to the increased strength and rigidity, the morphology (shape and structure) of cells is preserved.

Experimental procedure

One of the objectives of this work is to achieve real-time monitoring of biological processes with a high temporal resolution. We performed harmonic measurements using SNT with tips inserted in a microfluidic device. As a result, we could keep the actuating and sensing elements of the MEMS device in air for better performance by limiting the liquid insertion with only the tips. Using phase lock loop (PLL), a lock-in amplifier rode the sensing arm at resonance to characterize mechanical properties of a cell while compressing arm was squeezing the cell between SNT tips.

First, the microfluidic device was filled with liquid, e.g. culture medium or Phosphate Buffered Saline (PBS), by applying negative pressure to the outlet using the pump (Fig.

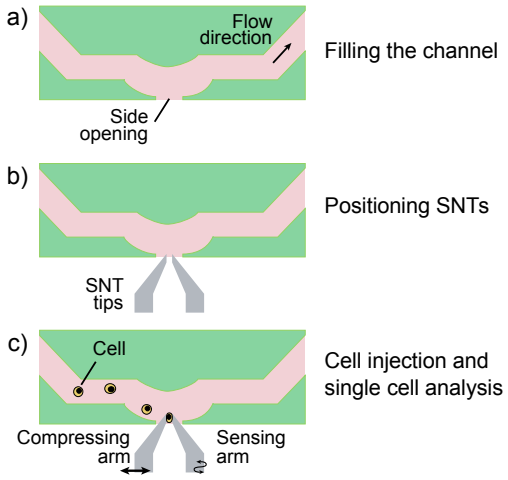

Figure 2: *a) The channel was filled with a solution, e.g. PBS. b) Then, tips were inserted via side opening. c) After injection, a cell was captured and analyzed. The vacuum pump was used to repeat this step with different cells.*

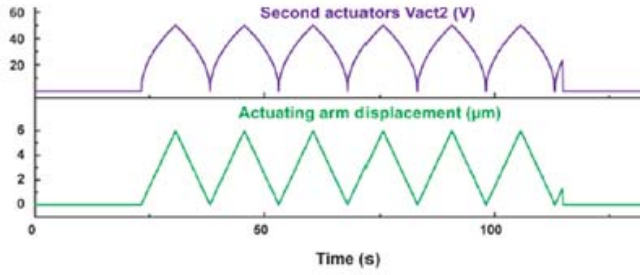

Figure 3: *To obtain a linear displacement (green) a square root signal had to be applied on the actuator.*

2a). Then, SNT tips were positioned at the side opening of the channel using the self-positioning of the system [8]. LabVIEW program kept the sensing arm vibrating at its resonance frequency while moving the motorized stage. A sudden shift in the resonance frequency was detected when the sensing arm touched the PDMS device. The nanometric steps of the stage protected the PDMS walls to damage the SNT tips. Using this method, the precise position of the side opening and the air-liquid interface could be detected. As a result of this positioning step, SNT tips were inserted in the channel via the side opening (Fig. 2b). Finally, a cell solution was injected in the inlet and the vacuum pump was used to bring cells in the close vicinity of the side opening. When a cell was right below the SNT tips, the flow was stopped and the motorized stage was used to locate the cell between the tips. At this point, the compressing arm started moving to decrease the gap between the tips and the cell response was detected using the sensing arm's sensor readings (Fig. 2c). Obtaining linear compression required a square root signal applied on the compressing arm (Fig. 3). To capture another cell, the tips were brought to their initial position and the pump created a flow to wash away the analyzed cell bringing another cell to the close vicinity of the side-opening within seconds.

RESULTS AND DISCUSSION

To perform single cell analysis, the air-liquid interface had to be stable throughout the measurements. Therefore, first, we tested the stability of the proposed system without any cells captured between the tips that were inserted in the solution (blue line in Fig. 4a,b). A potential difference was applied on the compressing arm actuator to obtain linear displacement (Fig. 4c). Neither the resonance frequency nor the amplitude was affected by the compression. Moreover, the results show stable characteristics throughout the experiment. Performing the same experiment with a fixed cell captured between the tips, we could detect the effect of compression clearly on the resonance frequency and the amplitude (red line in Fig 4a,b). The resonance frequency was increasing while the tips were compressing the cell. Although mechanical characterization could be performed in few tens of seconds, the microfluidic device allowed up to 10-minute testing without compromising the stability (Fig. 4).

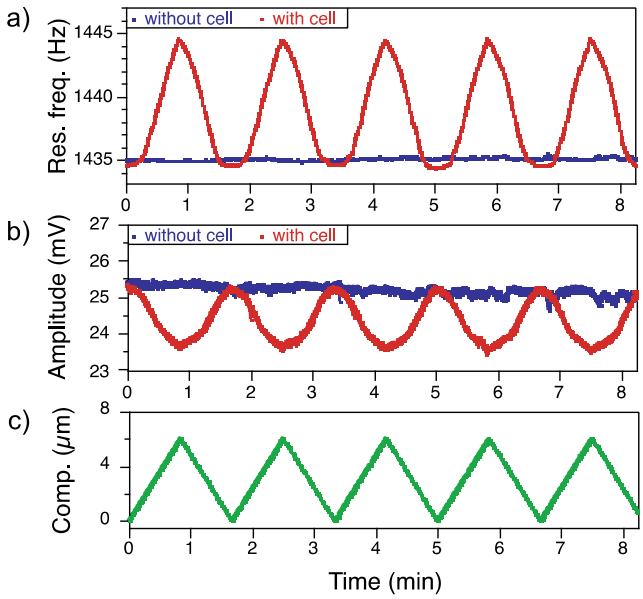

Figure 4: *SNTs were capable of detecting a) resonance frequency and b) amplitude in real-time. A fixed breast cancer cell (red) was compressed (as shown in c) repeatedly (100-s periods) to test the stability and compared with a no-cell control (blue). All x-axis corresponds to time in minutes.*

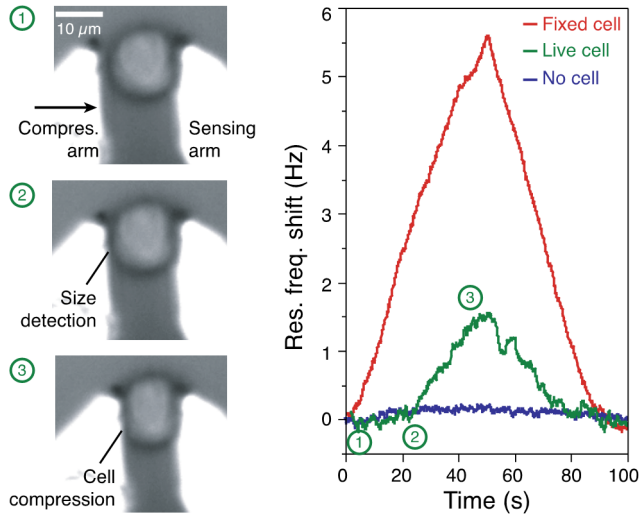

Figure 5: *Applied compression cycles resulted in smaller response frequency changes for live cells (red) compared to fixed cells (green). A cell response deviated from no-cell (blue) control when the cells started to be compressed.*

The setup controlled by the LabVIEW program allowed the real-time measurements to detect the diameter of a captured cell. Although only the sensor output was enough to detect the cell diameter, we confirmed with a camera and image processing tools. Using a camera to monitor the cell compression showed that the resonance frequency of the sensing arm stayed constant (Fig. 5-1,2) until the cell started to be squeezed by the compressing arm (Fig. 5-2,3). Knowing the gap between the SNT tips and the

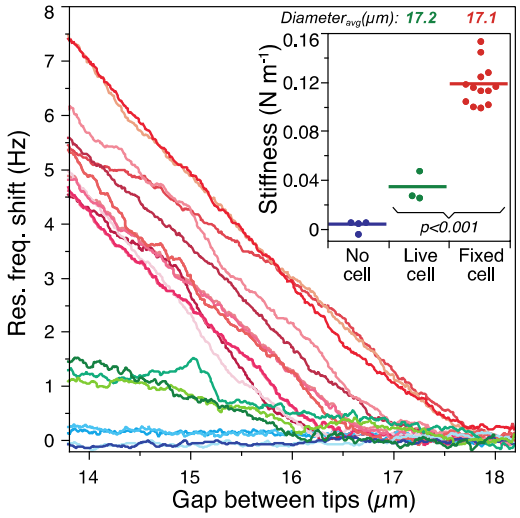

Figure 6: The gap is equal to the diameter of the analyzed cell when frequency response shifted from initial condition. The measured stiffness at 10% compression was significantly different (p<0.001, inset) between live (green) and fixed cells (red).

displacement due to the actuator, we could detect the cell diameter by observing the resonance frequency shift. The cell diameter corresponded to the gap between tips when resonance frequency started increasing.

We compared the mechanical response of fixed and live cells by applying linear compression cycles of 100s period. The control experiment (no cell) showed no increase in the resonance frequency. Therefore, we can conclude that a frequency shift of ~5.5Hz for a fixed cell and ~1.5Hz for a live cell were solely due to the analyzed cells' mechanical response (Fig. 5).

Performing the proposed cell analysis cycles for several times, we could observe differences between live and fixed cells as expected. Fixed cells showed much higher resonance frequency shift (red lines in Fig. 6) when compared to the live cells (green lines in Fig. 6). No cell control experiments showed the effect of cell response clearly. SNT was modeled with damped harmonic oscillator [8] and the sensor readings could be used to calculate the detected stiffness. According to the results, compression at 10% of the cell diameter gave significant differences (*t-test*, p<0.001).

CONCLUSIONS

Early detection of CTCs in blood is vital to start therapeutic actions before tumors develop resistance to treatments. Early detection requires rapid, cheap, easy-to-use yet reliable methods. Therefore, non-labeled methods, alternative to immunoaffinity-based approaches, have to be developed. CTC detection and characterization based on mechanical properties is a promising alternative. Techniques performing mechanical characterization suffer either from low-throughput or low information content. Here, we showed how we can benefit from the MEMS technology to improve the efficiency. Combining the handling, sensing and manipulation elements on a single device and integrating this device with microfluidics minimize the time/effort for the setup. Moreover, automating the process allows even non-specialist users to perform the mechanical characterization. Preparation of the proposed setup and characterization of cells can be performed within minutes. Demonstrated process is the first step towards a non-labeled method for multi-parameter mechanical characteristic of cell targeting early detection of metastatic CTCs.

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