35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Integrated MEMS platform with silicon nanotweezers and open microfluidic device for real-time and routine biomechanical probing on molecules and cells

Nicolas Lafitte[1], Hervé Guillou[1,3], Momoko Kumemura[1,2], Laurent Jalabert[1], Teruo Fujii[2], Hiroyuki Fujita[2] and Dominique Collard[1,2]

Abstract— **This paper describes an integrated biomechanical platform for real-time molecular or cellular assays. This platform is composed of silicon nanotweezers to manipulate the biological samples and an open microfluidic to handle solution and reactive agents. The tweezers are fabricated by standard Silicon-On-Insulator based micromachining processes (2 masks $+1$ additional mask for special tips) and integrate actuator, trapping tips and sensor. The microfluidic device is produced from common polydimethylsiloxane (PDMS) micromolding and integrates active valves for controlling the biological medium. Combining both technologies, a versatile experimental setup, built up in an enclosed space (< 10 cm^3), enables direct interrogation of molecules or cells in solution.**

The silicon nanotweezers sense slight biological modifications of the trapped molecules or cell by monitoring the mechanical resonance response, which keeps a high Q factor (over 20) in liquid. Biomolecular assays (molecule trapping and enzymatic reaction kinetics) as well as characterizations of cells are reported here. The system provide molecular level resolution and is sensitive enough to capture cell biomechano-transduction activities. Moreover as the system is handy, it may be an easy, fast and quantitative alternative to existing methods.

I. INTRODUCTION

In the field of molecular biology, a variety of remarkable techniques such as fluidic. electric, magnetic and optical traps have been developed to trap and directly interrogate molecules [1]. Such techniques interact mechanically with the molecule and measure the forces related to the structural configuration of the molecule. These have proven the relevance of mechanical characterizations in biology and we have thus developed a MEMS (Micro Electro Mechanical Systems) device in order to perform mechanical assay at the molecular and cellular scale. MEMS are appropriate because they are integrated, monolithic, cheap and can be engineered for specific applications.

Hereafter the silicon nanotweezers are introduced. The Sectionis completed with the description of the complementary open microfluidic which allows the control of the biological medium. In Section III, are demonstrated the trapping of DNA molecules, the real-time monitoring of bioreactions on DNA molecules as long as the application to cell characterizations. The paper concludes summarizing the capabilities of the developed platform and perspectives (Section IV).

[1]are with the LIMMS/CNRS-IIS (UMI 2820), Institute of Industrial Science, the University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan.

[2]are with the Institute of Industrial Science, the University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan.

[3]is with the Institut Néel, CNRS and Université Joseph Fourier, Grenoble, France.

E-mail: lafitte@iis.u-tokyo.ac.jp

II. DESCRIPTION OF THE PLATFORM

The experimental platform is composed of two complementary parts. The sensing tool is the SNT which are capable to: 1) trap molecules or cells, 2) mechanically stimulate them and 3) sense slight changes of the mechanical model. The second part is an active microfluidic device developed for bio-experiments in small amounts of solution and controlled conditions.

A. Silicon nanotweezers (SNT)

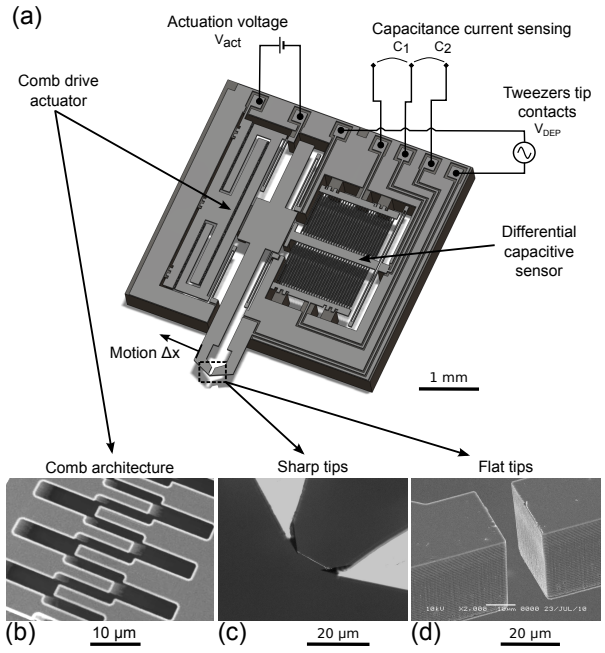

Fig. 1. Silicon nanotweezers for bio-electro-mechanical characterizations of molecules bundle. Dimensions: 4.5×4.5 mm. (a) 3D schematic view of the tweezers. The mobile electrode is electrostatically actuated by V_{act} and the motion displacement changes the capacitances C_1 and C_2. (b) Close view on the electrostatic comb-drive actuator (SEM image). (c) Sharp tips in between a bundle of fibronectin molecules is trapped (Optical microscope image). (d) Flat tips for cell manipulation (SEM image).

1) Working principle: Figure 1 shows a three-dimensional illustration of the device. It consists of 2 tips that can be

978-1-4577-0216-7/13/$26.00 ©2013 IEEE

fabricated sharp to address long macromolecules or flat to address whole cell (Figure 1(c) and (d)). One tip is fixed and the other one can be moved with an electrostatic actuator. The motion of the tip is measured using two capacitances with gaps that vary in proportion to the tip displacement. Therefore with an appropriate electronic read-out (especially pA-current amplifiers and a lock-in amplifier), the displacement or the velocity of the moving tip is inquired in real-time and continuously. The microfabrication and the instrumentation of the SNT are reported in [2].

2) Sensing method: The mechanical characteristic of the trapped molecules (rigidity and viscous losses) are measured in real-time by monitoring the resonance frequency of the system SNT+molecules (Figure 2(a)). When the sensing tip is in contact with the sample, the resonance response changes according to the added rigidity and the added losses of the sample (Figure 2(b)). The SNT is identified as a 2^{nd}-order oscillator with M the mass of the moving tip, k the mechanical stiffness of the suspensions and ν the viscous losses mainly due to the medium (air and liquid). The mechanical contribution of the sample is extracted from the shifts of the resonance frequency and amplitude, and the model of the SNT (Figure 2(c)).

The monitoring of the resonance of the system with a Phase-Locked-Loop (PLL) algorithm enables to follow evolutions with time resolution down to 300 ms. The resolution in stiffness and in viscous losses attains respectively 0.2 mN.m^{-1} and 0.05 µN.s.m^{-1}, limited by the electronic noise of the instrumentation. The sensing method and the model equations are more deeply detailed in [3].

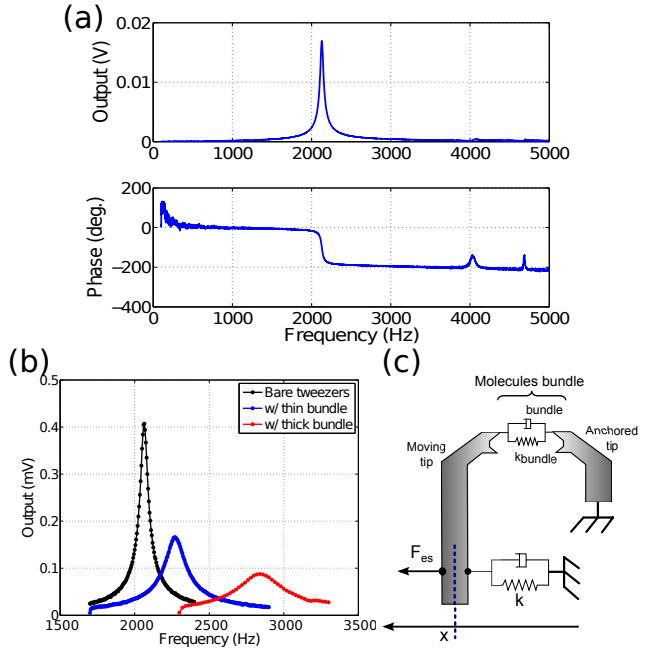

Fig. 2. Model and sensing principle of the SNT. (a) SNT frequency response with the resonance peak. The response fits with a 2^{nd}-order mechanical oscillator model. (b) Resonance responses without and with 2 different DNA bundles in air. (c) DNA bundle mechanical characteristics are identified from the original model of the SNT.

B. Open and active microfluidic

A complementary microfluidic have been developed for experiments with small amounts of solution (< 5 µL) and control of the evaporation. Therefore the microfluidic device is based on the integration of an open chamber and the integration of pneumatic valves nearby the reaction chamber for fast commands of the inlets and the outlet (Figure 3).

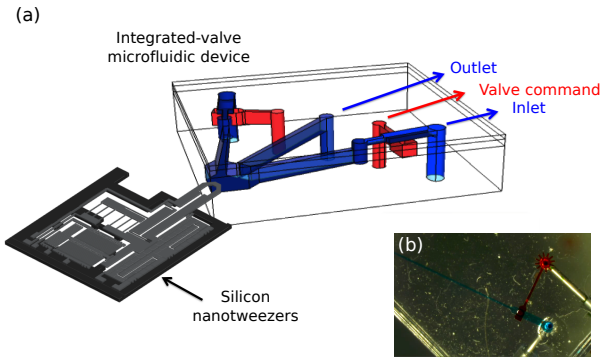

Fig. 3. SNT-integrated microfluidic setup. (a) 3D schematic: the microfluidic device in PDMS has an open cavity (200 µm height and 4 mm large) for the insertion of the tweezers' tips and 2 active pneumatic valves for controlling the solution inlets. Flow channels and the open reaction chamber are in blue and control channels in red. (b) Microscope photo (top view) of a pneumatic valve, i.e. the cross-section of the red control channel and the blue flow channel.

Active valves are fabricated using crossed-channel architecture [4]. The device is made of PDMS sealed layers and a glass substrate. A thin layer is produced to implement the controllable flow channels (around 16 µm height), when a thick layer is fabricated for the control channels and the open reaction chamber (200 µm height). The two layers are produced by replica molding from two masters and sealed together. More details about the fabrication and the molding are provided in [3], [5].

The valve membranes are formed where the control and the flow channels (600 µm wide each) intersect orthogonally (Figure 3(b)). The red-colored liquid controls the blue-colored liquid. When a control pressure of 100 kPa is applied to the lower channel, the intermediate elastomeric membrane deflects upward and closes the upper channel stopping the flow. When the pressure is released, the blue liquid flows in the direction of the open chamber. Finally combined with a convenient flow pressure (< 10 kPa), the response time of the device is fast enough (< 10 ms) to precisely fill the reaction chamber.Furthermore, the valve stops tightly and hermetically the channel such as the open chamber does not overflow after closing the valve.

III. BIO-CHARACTERIZATIONS ON MOLECULES AND CELLS

In order to demonstrate the capabilities of the SNT platform for performing bio-mechanical sensing on bio-samples, three types of experiments are illustrated here:

1) The trapping of DNA molecules,
2) The real-time monitoring of molecule binding on DNA,
3) And the assays on biological cells.

A. Trapping of DNA molecules

1) Material and methods: Double-stranded λ-DNA (48.5 kbp, 16 μm length, from Takara Bio Inc.) was used in the experiments, diluted twice with *Milli-Q* water and flowed into the microfluidic. DNA molecules are trapped by dielectrophoresis (DEP) which allows the orientation of DNA strands in an electric field according to the frequency [6].

Firstly, the tweezers tips are brought to the surface of the DNA solution from the open side of glass slit (Figure 4). Then an AC voltage is applied between the tips (1 MHz, 20 V_{PP}) such as DNA molecules extend and are trapped in between the two tips of the SNT (as shown in Figure 1(c)).

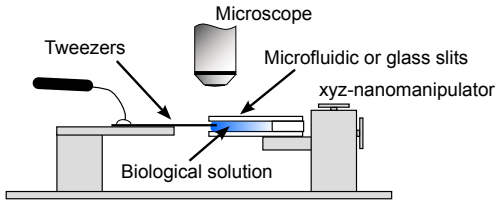

Fig. 4. Side view schematic of the experimental setup for molecular reactions (molecules trapping and molecular interactions).

2) Results: The resonance response of the system is monitored during the whole experiment. During the trapping proceeds, the resonance frequency increases (from 2491.7 Hz to 2492.4 Hz) due to the addition of the rigidity of the DNA bundle k_{bundle}. At the same time, the amplitude tends to decrease (from 23.65 mV to 22.95 mV) as the bundle brought losses to the system. The Figure 5 focuses on the increase of the stiffness and the losses in the bundle during trapping. Finally knowing the stiffness of a single λ-DNA molecule (30 $\mu N.m^{-1}$ when extended [7]), we deduced a trapping rate of 0.9 molecule/second.

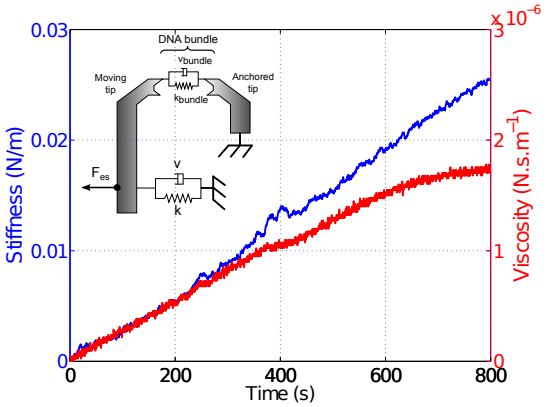

Fig. 5. SNT & bundle rheological model. The graph shows the evolution of the model parameters during trapping. Stiffness and losses are deduced according to the dynamic properties of the bare system.

B. Real-time monitoring of DNA intercalators reactions

1) Material and methods: Ethidium bromide (EtBr) is an intercalating agent commonly used as a fluorescent tag to detect nuclear acids in molecular biology. Such type of intercalation reactions interfere with biochemical processes by modifying the structure of the ds-DNA [8].

After the DNA trapping, EtBr solution is pipetted in a glass slit and the SNT probes are introduced with the DNA bundle into the reaction cell (Figure 6(a)). The experiment is performed twice with two different concentrations of ligands (2.5 μM and 250 μM).

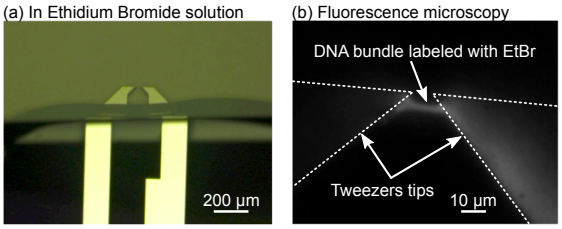

Fig. 6. (a) Image of the SNT when immersed into EtBr solution (top view). (b) DNA bundle examined by fluorescence microscopy.

2) Results: Likewise during the DNA trapping, the measured resonance frequency increases and the amplitude decreases during the experiment. The Figure 7 shows the corresponding stiffness and losses brought by the intercalation of molecules inside the DNA bundle at the two different concentrations. Both curves show a similar temporal evolution of the bundle properties with the molecule binding. However, close-up view on the kinetics shows rising time independent to the ligand concentration, pointing possible competitive processes between adsorption and dissociation inside the DNA bundle [9].

The bundle is controlled under fluorescence microscopy in order to confirm the labeling of the DNA (Figure 6(b)).

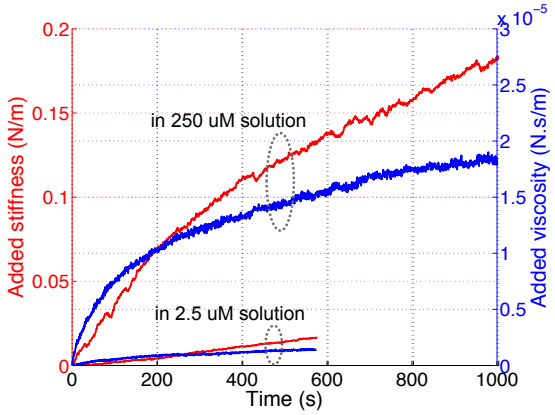

Fig. 7. Evolutions of the bundle stiffness (in red) and viscosity (in blue) during molecule binding on the DNA. Experiments were performed with 2 different concentrations (2.5 μM and 250 μM EtBr concentrations).

C. Experiments on cells

1) Material and methods: For the experiments on cells, SNT are developed with two integrated actuators allowing larger stroke of the gap and enabling larger deformation/compression of the cell [10], [11].

The RPE-1 cells are cultivated until 50% of confluence and trypsinized [12]. The cell are suspended into a micro petri dish designed to limit the amount of fluid. The SNT tips are open and positionned onto a cell (Figure 8). The surface is approached in controlled manner to within 100 nm

by monitoring the amplitude of the resonance. To avoid any interaction with the surface the SNT are then lifted 1 µm above. The SNT are then closed by reducing the actuation voltage on the large actuation arm. The large actuation arm is then alternatively opened (Figure 9(c)) and closed (Figure 9(b)) to mechanically stimulate the cell while the mechanical properties of the system are recorded.

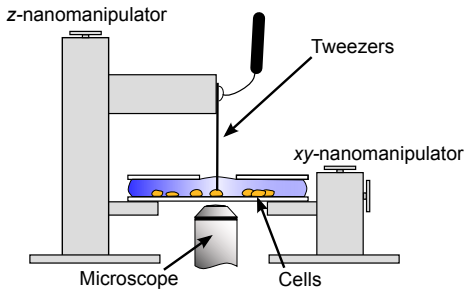

Fig. 8. Experimental setup integrated on a fluorescence microscope and with nanomanipulators for the addressing of cells.

2) Results: The graph of the Figure 9 shows the recording of the mechanical properties on a long time scale. The stiffness changes on a long time scale suggesting that the cell may have triggered signaling pathways to reinforce the actin cortex. The dissipation changes little. The force shows a strong viscous behavior both at closing and opening. This viscous behavior is not observed in the dynamic properties. We hypothesize that our system is able to probe the mechanical properties on different time scales. For time scales on the order of the resonance period (< 1 ms) only fast processes are probed these maybe associated with single actin filaments [13]. Slower time scales are directly probed by the force measurements. At these time scales the hydrodynamic forces associated with the flow of the cytoskeleton are relevant. Further investigations are being undertaken to validate these hypothesis.

IV. CONCLUSION

The developed SNT platform is demonstrated as a versatile experimental setup with high sensitivity for real-time characterizations of biological molecules and cells.

We have successfully measured the kinetics of bioreactions on DNA and mechanical properties of a single cell trapped in between the SNT tips. We are able to probe different type of samples by engineering the tips (shape, gap and stroke) and different time scales associated to the molecular and cellular processes. The system may be an easy and fast alternative to existing methods to perform quantitative bio-mechanical assays.

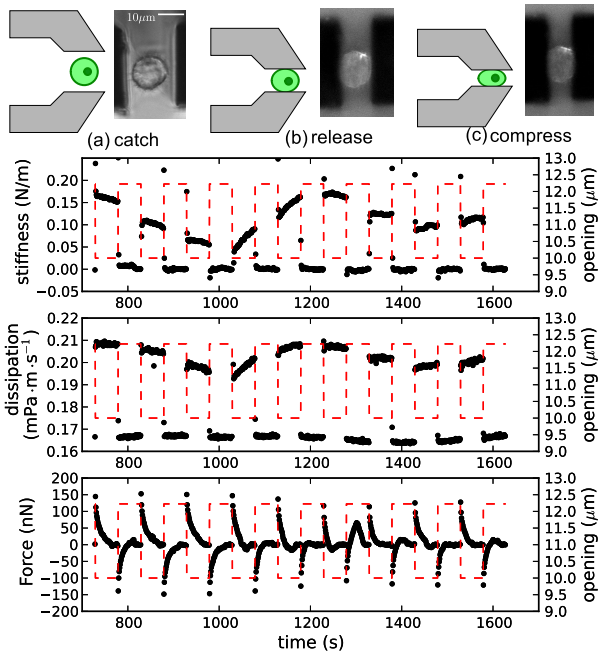

Fig. 9. Repeated compressions and release of a single cell in suspension. (a) The initial diameter of the cell is 13 µm. (b) Schematics and fluorescence image of the cell in compressed state; (c) Of the cell in released state but still hold by the SNT. The upper arm is used for the mechanical stimulations and the lower arm senses the stiffness and the dissipation (dynamic properties at the resonance frequency) and the absolute force (static). The opening of the SNT is plotted as a dashed line.

REFERENCES

[1] C. Bustamante et al., "Grabbing the cat by the tail: manipulating molecules one by one." Nat. Rev. Mol. Cell Biol., vol. 1, no. 2, pp. 130–136, Nov. 2000.

[2] C. Yamahata et al., "Silicon Nanotweezers With Subnanometer Resolution for the Micromanipulation of Biomolecules," Microelectromechanical Systems, Journal of, vol. 17, no. 3, pp. 623–631, 2008.

[3] N. Lafitte, "Modeling and control of MEMS tweezers for the characterizations of enzymatic reactions on DNA molecules," Ph.D. dissertation, Université de Franche-Comté, France, FEMTO-ST/LIMMS, Apr. 2012.

[4] M. Unger et al., "Monolithic microfabricated valves and pumps by multilayer soft lithography," Science, vol. 288, no. 5463, p. 113, 2000.

[5] N. Lafitte et al., "An open microfluidic device with active valves for accurate trapping of DNA by silicon nanotweezers," in 14th Int Conf. on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS2010, Groningen, The Netherlands, 2010, pp. 1865–1867.

[6] M. Washizu and O. Kurosawa, "Electrostatic manipulation of DNA in microfabricated structures," Industry Applications, IEEE Transactions on, vol. 26, no. 6, pp. 1165–1172, 1990.

[7] S. Smith et al., "Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules," Science, vol. 271, no. 5250, p. 795, 1996.

[8] I. D. Vladescu et al., "Quantifying force-dependent and zero-force DNA intercalation by single-molecule stretching," Nature methods, vol. 4, no. 6, pp. 517–522, Jun. 2007.

[9] N. Lafitte et al., "Real-Time sensing of molecule binding on DNA with silicon nanotweezers," in 15th Int Conf. on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS2011, Seattle, The USA, 2011, pp. 389–372.

[10] M. Kumemura et al., "Integrated MEMS platform with silicon nanotweezers and open microfluidic device for molecular and cellular biomechanical assays," in Micro Electro Mechanical Systems (MEMS), 2011 IEEE 24th Int. Conf. on, Cancun, Mexico, Jan. 2011, pp. 67–70.

[11] H. Guillou et al., "Silicon nanotweezers for easy cell biomechanics measurements," in NMC 2013, 10th Int. workshop on Nanomechanical Sensing, Stanford, California, USA, May 2013.

[12] Q. Tseng et al., "Spatial organization of the extracellular matrix regulates cell-cell junction positioning." Proceedings of the National Academy of Sciences, vol. 109, no. 5, pp. 1506–1511, Jan. 2012.

[13] L. Deng et al., "Fast and slow dynamics of the cytoskeleton," Nature Materials, vol. 5, no. 8, pp. 636–640, Jul. 2006.