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Electron beam induced fine virtual electrode for mechanical strain microscopy of living cell

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ABSTRACT

We have demonstrated nanomechanical applications using physicochemical and electrochemical phenomena of inverted-electron beam lithography (I-EBL), which induced *in-situ* two-dimensional (2-D) processing on wet samples and a living cell after the EB was stoppted in a 100-nm thick SiN membrane. The incident EB generates a virtual electrode and then this induces electrochemical and electrokinetic phenomena around the scanning trajectory. The I-EBL processing has a 120-nm resolution in full-width-at-half-maximum (FWHM) at the deposited line-and-space pattern in 10 mM 3,4-ethylenedioxythiophene (EDOT) diluted water solution. The virtual electrode also causes a electrokinetic local repulsive force with $<\sim 1\,\mu m$ resolution toward the negatively charged nanoparticles, and the 2-D scanning of the EB allows 2-D actuation of the nanoparticles dispersed in a pure water solution. The virtual electrode also induces local detachment of adherent nanoparticles and focal adhesion of a living cell from the SiN membrane in a saline solution, probably due to both electrokinetic and partly chemical protein denaturation processes. The local detachment of a living cell is utilized to investigate spatio-temporal distributions of intracellular elastic strain as mechanical strain microscopy (MSM), which represents mechanical connectivity in the intracellular structure. This MSM should provide visualization of the location of the force generation in the cell.

1. Introduction

The primary role of spatio-temporal mechanochemical processes at both mechanical force generation at cell migration and mechanotransductions of environmental changes is as a self-regulating system and it is performed using a variety of nano scale structures on protein molecules system. Migrating cell senses difference of environmental mechanical properties [1,2] and responds to them to regulate own migrating direction. The migration force generation is performed by construction of nanoscale actin fiber network and focal adhesions, [3] and the actin filament bilaterally transmits mechanical information as balancing tension of fiber network and adhesion molecules. [4,5] Therefore, to understand the system dynamics of biomechanical behaviors in more detail, we have to employ a biomanipulation technique which requires physicochemical manipulation and to cope with the needs for both accessibility to any spatio-temporal samples and hundred-nanometer resolution of the biomanipulation technique for mechanochemical activities in living cells. Microprobing and manipulating devices have contributed to controlling such local phenomena of biomolecule systems in living cells [5–11] and spatio-temporal cell pattern [6–8,12–17]. Such high resolution manipulation of biological dynamic systems is of both fundamental and practical importance in system analysis and biomedical applications. Furthermore, high speed approaching of the manipulator probes and a parallel processing of large number of the target samples are important to improve the analysis throughput. However, previous nano and micro biomanipulations based on mechanical, optical, and electrokinetic techniques had not satisfied these requirements simultaneously.

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In-situ micro and nanoscale mechanical stimuli and spatio-temporal measurements of mechanical forces have been utilized to understand how the mechanotransduction works and the cell responses to the mechanical stimuli. Optical probes using a focused laser have been used successfully to demonstrate three-dimensional various intracellular and subcellular stimuli *in-situ*; examples include, a multiphoton induced ablation of a single actin stress fiber for a mechanotransduction study [5], trapping forces for mechanical studies of molecules [18,19], and direct cutting of neurons into lengths of a few micrometers [11]. In particular, combination studies of a single stress fiber cutting and simultaneous traction force cytometry elucidated the role of stress fibers for the mechanotransduction and cell migration [5]. Mechanical cytometry with more precise *in-situ* mechanical stimuli to each mechanical components; examples include, focal adhesion and stress fiber, should provide visualization of mechanical connectivity and mechanical transmission in molecular scale network, however the resolution was limited to a sub-micrometer level by light diffraction.

In other hand, electrochemical reactions are also significantly useful method for two-dimensional (2-D) mechanical stimuli to single adhered cells. Surface modification with electrochemical reactive surfactant could locally control cell immobilization and detachment by using pre-patterned electrode [12–14] and light induced-type surface modification [15] at the cell culture surface to analyze cell motility and tissue engineering [6–8,12–14,16,17]. A large number of target cells and spheroids can be applied to the simultaneous parallel analysis of biomechanics and tissue engineering in large area, which are an advantage to achieve high throughput cell analysis. The electric properties of the surface and the applied electric fields affect to the resolution and influences of the manipulation; therefore, the formation of the electrical field was considered carefully in the electrode design. Laser induced electroconductive spatial patterns have been used as a virtual electrode on an amorphous silicon layer [20,21]. The combination of the virtual electrode and an opposing counter ITO electrode offers the advantage of dynamically locating an arbitrarily shaped electric field for dielectrophoresis, although the virtual electrode diameter must be >1.5 μm because of light diffraction [21]. Thus, light-induced surface modification and the virtual electrode are limited the spatial resolution in optical diffraction, although rapid and *in-situ* manipulations are useful techniques for cell science.

Here, we propose 2-D finer local detachment method for mechanical strain microscopy (MSM) on a single living cell using the virtual electrode which is induced by an electron beam (EB). We previously reported EB induced dynamical processing in water solution [22–25]. Our approach uses direct electron charging on a culturing surface by an EB. Fine focusing of even a low energy EB is well-known to be below tens-nanometer size which is significantly finer than the light diffraction limit. Such fine resolution has been often utilized for a wet sample observation using a scanning electron microscope (SEM) [26] and a transmission electron microscope (TEM) [27], an EB excitation assisted optical microscope for ultra-high resolution observation [28], and nanoprocessing using liquid precursor [29]. We also reported inverted electron-beam lithography (I-EBL) for 2-D micro patterning on single living cells in the culturing conditions [23] The EB of the I-EBL, which was irradiated through the SiN membrane as a culture substrate, enabled control of the electrokinetics to directly charge a negative electric potential into the interface of the liquid culturing medium and the cell culture substrate as shown in Fig. 1(a). This is applicable to the selection of any arrangement of the local detachment of a living cell *in-situ*. The principle of our approach was 2-D patterning of the virtual electrode on SiN membrane surface, and that had electrochemical phenomena to the surface immobilized charged molecules.

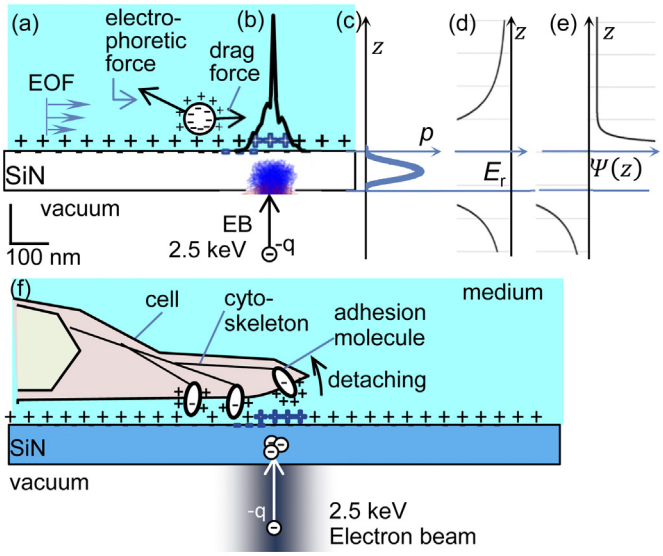

Fig. 1. Principles of a virtual electrode and mechanical strain microscopy (MSM) using the inverted-electron beam lithography. (a) Schematic image of virtual electrode due to an incident electron beam on a SiN membrane. The 100-nm thick SiN membrane was irradiated with the 50-nm wide EB of 2.5 keV. (b) Trajectories and horizontal distribution of the scattering primary electrons. (c) Vertical distribution of the scattering primary electrons. (d and e) Schematic graphs of the electric potential and electric field induced by the scattering primary electrons. (f) MSM of a single adherent living cell when locally detached at a lamellipodium using the spot EB irradiation. The adhesion molecule would be detached from the SiN due to Coulomb repulsion and an electrochemical reaction.

This paper reports the fine resolution of the I-EBL induced chemical reaction and the physicochemical and electrokinetic effects including electrostatic repulsion force from the EB pattern to the living cell and nanoparticles. The results of a biomechanical analysis demonstrate spatio-temporal distributions of intracellular elastic strain as MSM using the combination of a selective local detachment of a living myoblast and image tracking its transient response.

2. Principle of virtual electrode

Fig. 1(b) shows the simulated distribution of scattering primary electrons into SiN with a 100-nm thickness and density of 3.1 g cm^{-3} and H_2O with density of 1.0 g cm^{-3} from the vacuum side with a density of 10^{-3} g cm^{-3} at the beam acceleration voltage of 2.5 keV and beam radius of 50 nm. Our results of Monte Carlo simulation (using CASINO v2.48 [30]) for trajectories of primary electrons showed that the most of the primary electrons stop in the SiN membrane at 2.5 keV (Fig. 1(c)). [23,25] Although forward scattering electrons could reach near the SiN-liquid interface, the distribution profile of the scattered primary electrons (<50 eV) with the initial incident kinetic energy of 2.5 keV was kept inside the SiN membrane except for a very few transmitted electrons (Fig. 1 (c)). Although the small kinetic energy of the transmitted electrons is one factor influencing polymer deposition on the water-SiN interface [22], most of the electrical energy causes electrokinetic phenomena on or around the interface. The electric charges and small leakage of electric current principally affect the electrokinetic phenomena, for example, electroosmotic flow (EOF) [25] and Coulomb force [24].

The dielectric SiN membrane allowed accumulation of the electric charges of the primary electrons in a region about 100 nm wide (Fig. 1(b)). Thus, the beam spot formed a steep gradient of electric field in the SiN membrane and in the solution. Although the profile of Coulomb potential in the solution was restricted in depth by Debye shielding in the electrolytic solution, the electric potential remained in the vicinity of the SiN-liquid interface (Fig. 1(d and