FINE VIRTUAL CATHODE DISPLAY FOR BIOMOLECULES CONTROL AND CELL NANO SURGERY

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ABSTRACT

Electron beam (EB) induced fine virtual cathode (VC) on a 100-nm-thick SiN membrane has been developed using inverted-EB lithography (I-EBL). Here we present electrokinetic property of the VC for biological materials and instantly control their spatio-temporal responses using the VC. Negatively charged biomolecules (proteins, DNAs) respond to locally injected negative electrons of VC, and change their electrostatic interaction which affects the molecular aggregation/dissociation and adhesion/detachment in water solution. So, we call this technique virtual cathode display for biomolecules. This technique was applied to in-situ molecules patterning for mechanical strain microscopy and pinpoint transmembrane molecules delivery.

INTRODUCTION

Cell motilities and biomolecular behaviors have been often investigated on electrochemical and mechanochemical phenomena. To understand the biomechanical behaviors of living cell and molecules, we have to employ a fine biomanipulation technique which requires physicochemical manipulation with both accessibility to any spatio-temporal samples and hundred-nanometer resolution in living cells. Previous biomanipulation employed optical labeling, pre-designed electrode, and mechanical probes. [1]–[3] In contrast we have demonstrated non-labeling nanomechanical biomanipulation using physicochemical and electrochemical phenomena of inverted-electron beam lithography (I-EBL), which consists of inverted EB system and co-axial fluorescent microscope. [4] SiN membrane chip separated vacuum (lower-side) and water solution (upper-side), and incident electron with scanning induced a fine virtual cathode (VC) on the SiN membrane to control in-situ mechanical strain microscopy (MSM) on a living cell. [5]

FINE VIRTUAL CATHODE DISPLAY

I-EBL is in-situ EB lithography technique to wet samples through a 100-nm-thick SiN membrane with a low energy EB. [4] 2.5 keV EB of I-EBL stops its kinetic energy of electrons within the 100-nm thick SiN membrane, and the injected electrons in pinpoint generate negatively electrical charge (virtual cathode; VC) in dielectric SiN. The electrostatic potential on the VC could changes intermolecular electrostatic interactions, and allows controlling molecular aggregation/dissociation and adhesion/detachment (figure 1) at pinpoint and in-situ process. Moreover, the VC on the SiN membrane rapidly follows the incident scanning EB, therefore two-dimensional spatio-temporal pattern of the molecular responses can be instantly generated with any pattern design with EB resolution.

Figure 1 shows schematic view of the VC on I-EBL and its bioresearch applications. The VC display could pattern electrophoresis and electroosmotic flow on the VC display. The osmotic flow was directed inward to the VC on negative charged surface,[6] and electrostatic force generated electrophoresis of diluted nanoparticles in the solution. [6], [7] Moreover, electrochemical reactions were performed on the VC two-dimensional patterns. Quick scanning of the VC generated deposition of organic solute [5], [8] micro motions of surface coated polymers. [8] However, thermal generation induced by Joule heating and beam kinetic energy had enough small (<1 K at spot VC) for invasive biomanipulations with no heat damage, because water solution on the VC works as great heat transfer material. [5], [6] additionally, since low level EB leads impermeable primary electrons at the SiN membrane and less magnetic radiation, the VC would make less damages to the target molecules.

These physical and chemical effects on the VC could be applied for pinpoint cell manipulations of which pre-process labeling device design is unnecessary. Pinpoint detachment of an adhesive cell was performed for quantitative evaluation of intracellular mechanical strain distribution and intracellular mechanical connectivity. [5] .Additionally, here, we report more useful applications of VC, *e.g.* molecular patterning which adhered on the SiN membrane, and pinpoint transmembrane molecular delivery by electroporation.

MATERIALS AND METHODS

I-EBL and Sample preparation

2.5 keV EB of the I-EBL (lab made system of which EB column was mini-EOC of APCO) was scanned to the 100-nm-thick SiN (NT025C, Norcada) of which surface had adherent living C2C12 myoblast (Riken cell bank) in growth medium, phage T4 GT7 DNA (318-03971, Nippon gene) in Tris-EDTA buffer, and Goat anti-rabbit immunoglobulin G antibody secondary antibody labeled with fluorescent Alexa Fluor 546 (A-11035, Invitrogen) in Dulbecco's phosphate-buffered saline (-), respectively. Each surface of the SiN membranes was treated with positively charged polymer polyethylenimine water solution before above sample preparations.

The responses of these samples were recorded using simultaneous fluorescent microscopy

Pinpoint electroporation and transmembrane molecular delivery

The cell was cultured in growth medium (Dulbecco's modified Eagle medium, 10% fetal bovine serum, and 100U penicillin-streptomycin) with 10 μg/mL fluorescent

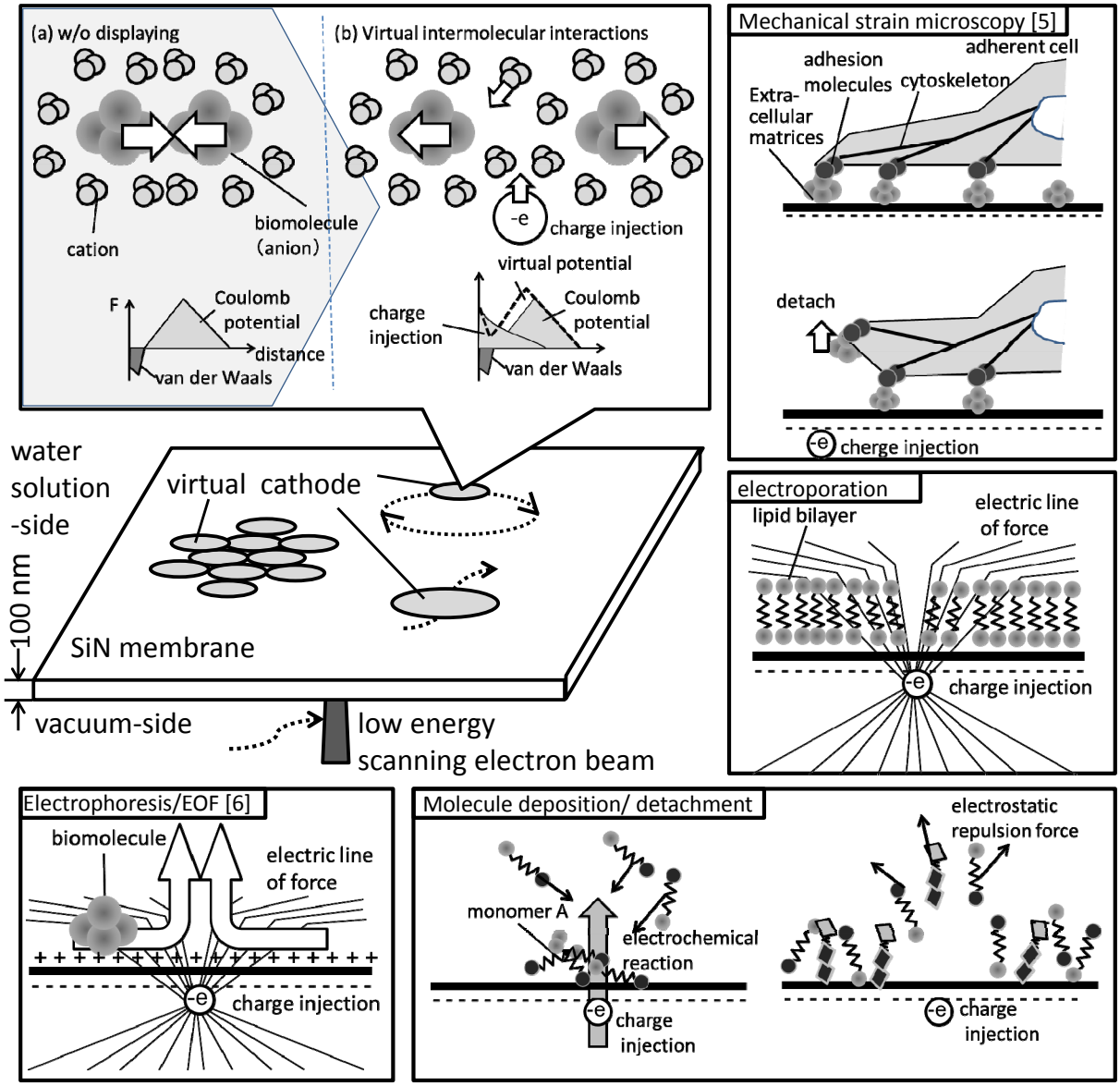

Figure 1: Principal of virtual cathode display using an inverted-electron beam lithography. The scanning electron beam is incident at bottom-side (vacuum-side) of a 100-nm-thick SiN membrane, and the electrical charge at the dielectric SiN displays instantly a virtual cathode (VC) on the upper-side of the SiN. VC could induce molecules response due to changing electrostatic interaction of the target molecules. Changing the molecules interactions control molecules aggregation/dissociation and detachment and electrophoresis at VCs. Such phenomena could be contributed to in-situ biomolecules manipulation and cell nano surgery.

dye PI during the experiment. A spot VC was applied on the cell membrane (cross mark at fig 2).

Molecular patterning

T4-DNAs was labeled with fluorescence YOYO-1 iodide (excitation/emission=491/509 nm, Y3601, Thermofisher). Surface adhered T4-DNA/YOYO-1 complex in TE buffer and Alexa Fluor-labeled IgG antibody in growth medium were separately treated with a VC pattern of raster scanning in rectangle region for 1 s, as shown in figure 3 and figure 4, respectively.

RESULTS AND DISSCUSIONS

Pinpoint electroporation and transmembrane molecular delivery

Since PI intercalation with intracellular double strand DNA/RNA increases the fluorescent intensity ~20-fold, dye PI pinpoint transmembrane delivery into the living cell was clearly confirmed at just around the VC as shown in figure 2. Local electric field by the VC would disturb lipid bilayer of the cytomembrane and increase permeability of the cytomembrane at the VC. Some cells after the pinpoint electroporation were recovered from the membrane pores and confirmed the viabilities.

Molecular patterning

VC induced modifications of electrostatic

intermolecular interactions had seen on the adherent molecules. Figure 3 shows that T4-DNA/YOYO-1 complexs in globule state were repelled from the scanning VC region. Fast scanning of EB made rectangular VC area, and Coulomb force by the VC would exceed the other adhesive molecular interactions then they gently detached from the VC area.

Furthermore, nonspecific adsorbed IgG antibody labeled with Alexa flour dye shows complex detachment responses at a square VC area. Real time observation of fluorescent imaging indicated ionic condition around the VC would be changed by electrophoresis at same time when the antibody detachment. (figure 4) Increasing fluorescent intensity before the detachment (at t=+0.0s) would be caused by modification of binding/dissociation constant due to ionic electrophoresis. During the scanning VC treatment, the fluorescent intensities were deceased due to both probable mechanisms of detaching the IgG/Alexa-Fluor complex and oxidation of Alexa Fluor dye.

Mechanical movements of DNAs and fluorescent responses of IgG antibody to the VC treatment suggested negatively charged molecules (consist of DNAs and IgG antibody proteins) could be removed from the display surface by the scanning VC. This probable hypothesis was supported by previous electrokinetic and Coulomb repulsion results. [6], [7]

Two-dimensional VC display

The scanning EB on the SiN demonstrated rapid two-dimensional VC pattern and perform easily to target the living cell and biomolecules on the VC display at pinpoint and in situ.

CONCLUSION

In summary, the VC display was instantly control the two-dimensional pattern of biomolecules and membrane permeability. This technique would be nano surgery tool for the nanobiotechnology applications.

ACKNOWLEDGEMENTS

This work was partly supported by KAKENHI 15H05511.

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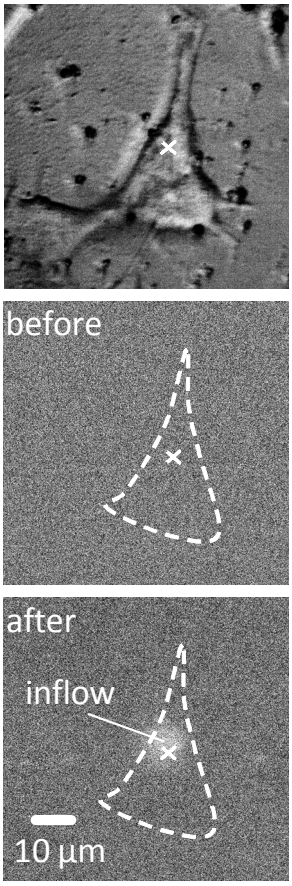

Figure 2: VC induced transmembrane pinpoint deliver of molecules. Bright image (top) and fluorescent images (middle, bottom) of a target C2C12 cell. White dotted line and mark with cross indicate the target cell and position of VC.

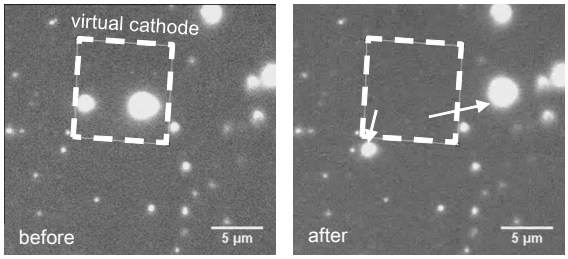

Figure 3: Phage T4 DNA detachment and repulsion from a square VC. Fluorescent images of YOYO-1 labeled DNAs. Globule state DNAs were adhered on the surface of the SiN membrane. White dotted line indicates area of VC.

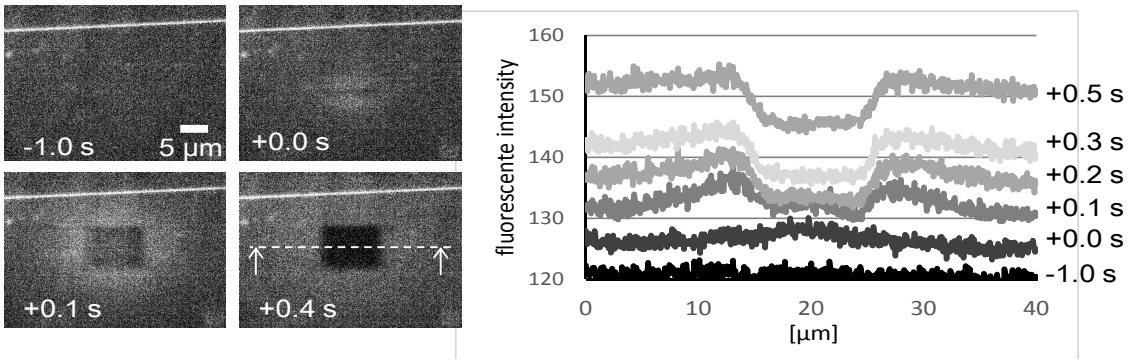

Figure 4: In-situ pattering of fluorescent-labeled IgG antibody A square VC generated instant pattern on the VC. Time response of fluorescent intensity on white dotted line (right) shows that initially increased and then molecular detachment occurred on VC. This indicates VC induces complex electrokinetic phenomenon during the detachment.

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