ELECTRON-BEAM INDUCED IN SITU SPATIOTEMPORAL NANOFABRICATION TOWARD INTRACELLULAR NANOROBOTICS

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ABSTRACT

Electron beam induced deposition on a live cell membrane was demonstrated as a nano stimulation for the cell response This, *in situ*, spatiotemporal nano fabrication would achieve a large scale analysis and a nano resolution of stimulation for individual mechanochemical receptor on the cell membrane. In liquid phase, electron beam induced chemical solution deposition was obtained through a SiN and SiO_2 nanomembrane window of a wet cell chamber. An electrical conductive polymer PEDOT could be polymerized using secondary electrons through the nanomembrane. The 5-10 keV electron beam through 15-100 nm thick SiN and SiO_2 nanomembrane successfully induced thin deposition pattern on the cell membrane in the cell culturing chamber.

KEYWORDS: Intracellular nanorobotics, Electron-beam induced deposition (EBID), Chemical solution deposition (CSD), PEDOT

INTRODUCTION

A mechanical and chemical response is result of changes of membrane traffic on a cell. The membrane traffic of ions and proteins through mechanochemical channels is physical carrier for signal transduction around the intracellular and intercellular system. In previous studies, therefore, artificial nano/micro environmental systems were applied for synaptic activities , mechano receptor , chemotaxis response [1-4]. Each devices employed a single chemical probing for environmental micro/nano stimulation, although the cellular system was nano scale molecular based interacting mechanisms in tens micrometers in diameter. In this study, we proposed the protein scale nano stimulation to modify the membrane traffic using electron beam induced solution chemical deposition of electrical conductive polymer PEDOT. Accelerated electron beam can be focused in nano scale diameter which is enough high resolution to observe protein [7-11] and fabricate protein scale nano pattern [12]. The electron beam induced chemical vapor deposition and chemical solution deposition in precursor materials. These depositions were also achieved on the opposite surface of the beam bombardment on the nanomembrane [5]. This nano scale electron beam energy transmitted through the nanomembrane could be utilized for a nano scale multi site stimulation. We applied this transmitted energy a stimulation probe for a live cell in a wet cell capsule which had a nanomembrane shielded window to establish a molecular scale control of cell membrane.

THEORY

Electro beam induces a surface chemical reaction to deposit precursor materials due to an emission of secondary electron form the surface. This is a result of equivalent energy of secondary electron to redox reaction. This electron beam induced chemical reaction could be applied for a modification of the cell membrane traffic as nano stimulation probes. We chose the precursor material 3,4-ethylenedioxythiophene (EDOT) which has high biocompatibility [6] for cell culturing. EDOT was well known precursor monomer for an electropolymerization of conductive polymer PEDOT.

When a low energy electron beam was irradiated through thin nanomembrane into the wet cell culturing chamber which was containing with EDOT solution (Figure 1). Figure 2 shows Monte Carlo simulation (CASINO [13]) of distri-

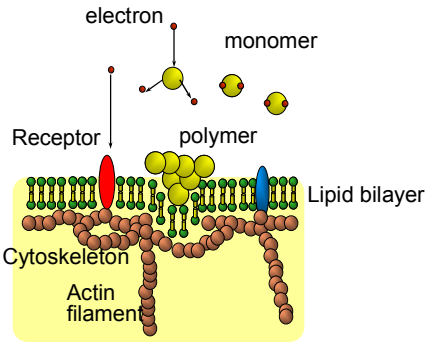

Figure 1 Schematic image of electron beam induced nanofabrication on a cell membrane. Polymerization of electro conductive polymer induced by electron beam bombardments would be utilized to stimulate mechanically the cell membrane and electrically.

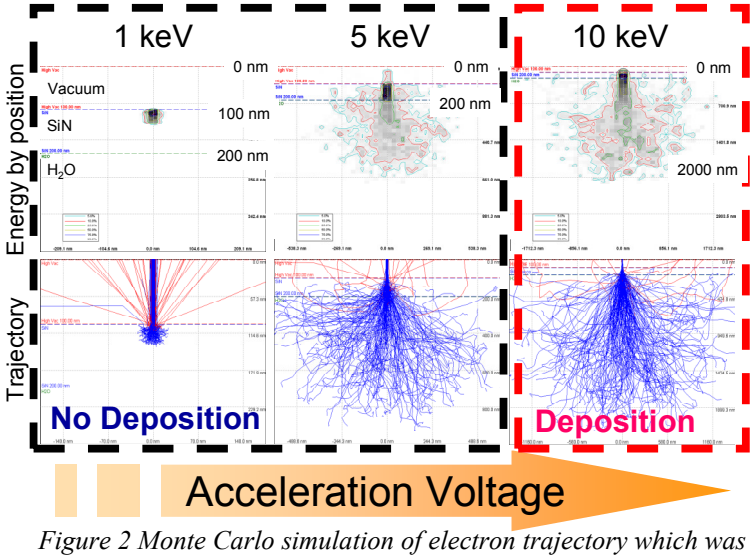

Figure 2 Monte Carlo simulation of electron trajectory which was penetrated into 1 atm H_2O through a 100-nm-thick silicon nitride membrane.

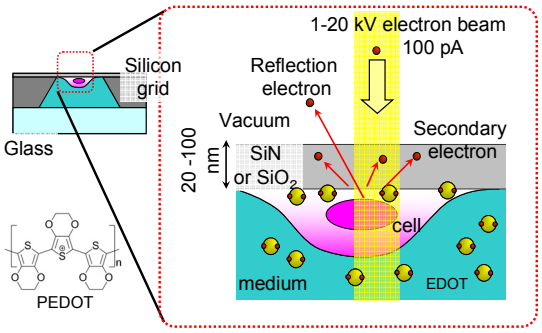

Figure 3 Schematic image of a wet cell wet capsule with a nanomembrane window. Living cells were cultured in the wet cell capsule with 20-100-nm-thick SiN and SiO_2. The culturing medium solved with EDOT.

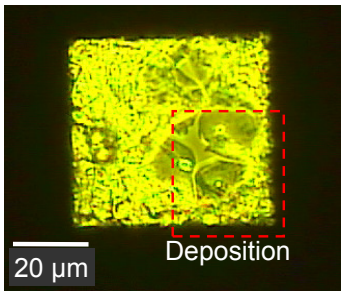

Figure 4 Bright field micrograph of electron beam induced PEDOT polymerization on the nanomembrane.

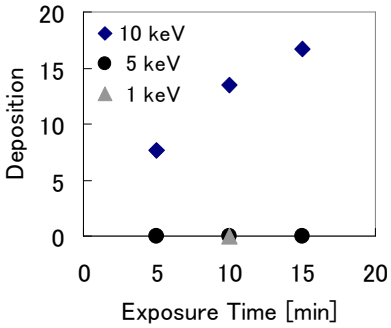

Figure 5 Deposition ratio on different acceleration voltage on 100-nm-thick SiN nanomembrane.

butions of primary electrons in the liquid phase water through a 100-nm-thick SiN nanomembrane. The energy distributions of the penetrating electron indicated that higher acceleration voltage over 5 keV could introduce enough high energy of the primary electron into the liquid water layer to induce the deposition.

To utilize the electron beam for *in situ* stimulus probe, the culturing medium and electron optics was separated with a nanomembrane as shown in figure 3. The irradiated electron beam was penetrated through the nanomembrane into the wet cell capsule. A limited electron range in 1 µm deep from the entry could reduce the serious damage to the cell nuclei function.

EXPERIMENTAL

Silicon grid with 20-nm-thick SiO_2 (SPI) and 100-nm-thick SiN (Norcada) nanomembrane were assembled in the wet cell capsule as shown in figure 3. Polymerization of electrical conductive polymer was demonstrated on the nanomembrane window of the wet cell capsule. Electrical conductive monomer 3,4-ethylenedioxythiophene (EDOT) which could be electrolytic polymerized to poly-EDOT (PEDOT) in biocompatible environment [6] was used for our demonstration. Myoblast C2C12 (Riken cell bank) was cultured on the 20-nm-thick SiO_2 nanomembrane windows. The wet cell capsule was filled with 10 mM EDOT (483028, Sigma) and conventional culturing medium; Dulbecco's Modified Eagle Medium (DMEM, 11995-065, Gibco) + 10% Fetal Bovine Serum (SV30014.03, HyClone). A focused 10 keV and <100 pA electron beam (VE-8800, Keyence) was scanned into cell culturing chamber containing with EDOT through the nanomembrane for 10 min.

The deposition properties were investigated with 1-20 keV beam on 100-nm-thick SiN nanomembrane. 10 mM EDOT diluted in DMEM was filled in the wet cell capsule. The deposited materials were analyzed using energy dispersive x-ray spectroscopy (EDS) (JEOL).

RESULTS AND DISCUSSION

The deposition of PEDOT was succeeded in the cell culturing medium using transmembrane electron bombardment (figure 4). The deposition was observed at over 10 keV acceleration voltage. The Monte Carlo simulation indicated that lower energy electron below 5 keV was blocked in the 100-nm-thick nanomembrane. This experiment results was acceptable to the simulation results. The deposited materials in figure 6 were detected as shown in figure 7. This EDS spectrum showed the deposition had carbon, sulfur, and some salt. Sulfur was component element of PEDOT. Some salt could be origin in the culturing medium.

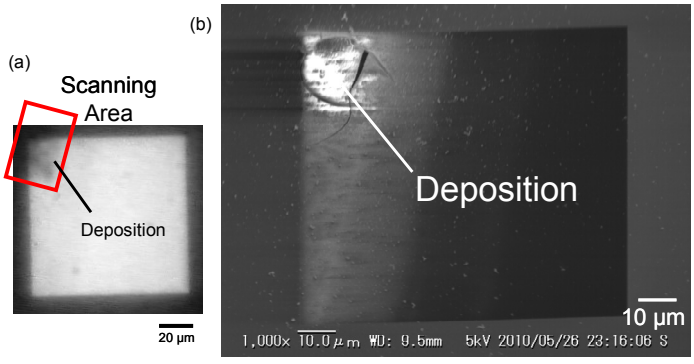

Figure 6 Electron beam induced deposition of PEDOT on a SiN nanomembrane. (a) Bright field image and beam scanning area. (b) SEM image of (a).

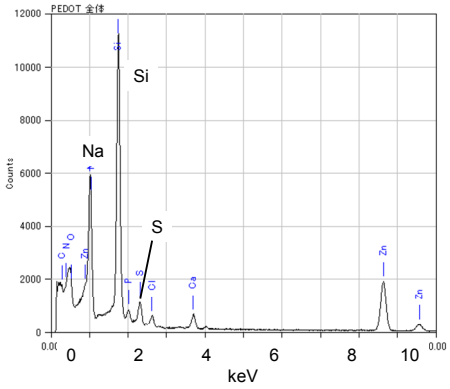

Figure 7 Energy Dispersive x-ray Spectroscopy of the deposition.

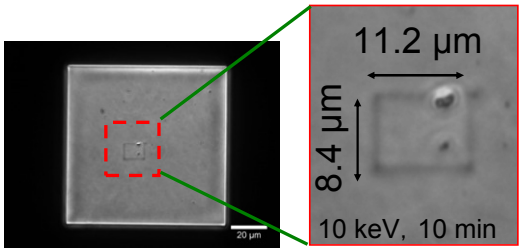

Figure 8 Micro patterning of deposition on a 100-nm-thick SiN nanomembrane

The patterning resolution was depended on the electron beam focusing. Figure 8 shows rectangular micro patterning of the deposition. The electron beam induced deposition had a micrometer resolution at our system. The simulation suggested that tens nanometer would be achieved using less than 10 nm in diameter of electron beam.

CONCLUSION

The electron beam induced oxidation-reduction reaction in cell culturing medium was confirmed. This *in situ* nano patterning and cellular nano stimulation would be utilized for real time analysis of cell membrane traffic and molecular function o intracellular protein systems. The electron beam has, in theoretically, less than nanometer scale wave length, and the electron range in water was also nano scale. Therefore this process could be applied to molecular level analysis for micro scale system, for example single spine reaction of neuron and cell migration on the nano membrane.

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