DIRECT CHEMICAL-COMPUTER INTERFACE
FOR LIVING CELL ANALYSIS

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ABSTRACT
An electron-beam lithography (EBL) was used as an in situ nano processing for a living cell analysis. A synchronized optics was containing an inverted EBL and an optical microscope. This system visualized real-time images of the EB-induced nano processing. We demonstrated the nano processing for a culturing cell with 50-300 nm resolution. Our system would be able to provide high resolution display of virtual environments. Our nano processing on a culturing living cell using an EB-induced reaction could directly manipulate the molecular chemical property of the wet environment. Moreover, theoretically, the EB had less than a nanometer scale wavelength, and the electron range in water was also hundred nanometer scale.

KEYWORDS: Inverted electron beam lithography (I-EBL), reaction-diffusion system,

INTRODUCTION
Direct connections on each different science and engineering; ie biophysics, biochemistry, information engineering, and control engineering, are absolutely necessary for reconstructing biophysical functions with tissue engineered prosthetic organs to recover from any disease. In this study, I propose that bio-physicochemical engineering based on computer engineering and control engineering to be a future self-organized biomedical engineering. We directly connect control engineering to biophysics and biochemistry via information engineering tools.

Biochemical reaction systems which formed spatial patterns in information pathways of cellular systems, the cell migration, and differentiations were fundamental to cell self-organization patterning and emergent systems. The biochemical reaction systems were modeled as the interactions and the spatial defuses of multiple chemical species. Turing pattern was often generated as a spatial pattern on reaction-diffusion systems using autocatalytic reaction, and also Turing pattern was observed on biological skin patterning. The reaction-diffusion systems in previous researches were assumed globally uniformed parameters of the reactions to generate Turing pattern. True biological reaction systems, however, contain local energy and species transfer. Therefore, the locally nonequilibrium reaction-diffusion systems was important to understand true biological reactions on self-organization patterning. A global control method and a direct chemical generator would contribute us a chemical-computer interface to directly control the self-organized reaction pattern based on a mathematical model at the real biochemical reactions.

In this study, we propose direct control interface of a local energy distribution on the reaction-diffusion system, and real-time observation of them to feedback the pattern. This interface is combined with an inverted electron beam lithography (I-EBL) and optical microscope in co-axial. The interface can control the reaction pattern in nanometer scale resolution. Cross-correlation analysis will provide us a transfer function of the biochemical reaction and cellular systems by using the electron beam input signals which contains control pattern, and its synchronized response. Turing pattern will be also controlled with a novel dynamic mode.

THEORY
Chemical reactions in a liquid phase were basic processes for the biological molecular systems which had even complex multistep cascade and dynamics. Protein molecule reactions promote nano scale movements, and also organization of macro structure and function of organs through by living cells motility and differentiations. Self-organization of spatio-temporal patterns by the chemical reactions and defuses were meaningful mechanisms to understand the organ and body design of living beings. These mechanisms of the self-organization could provide us constructing method of the tissue engineered organs. Therefore, control interface of the chemical reactions in nano liquid condition at room temperature was helpful tools for the researched of self-organization and tissue engineering.

We coupled principle of electron beam induced reaction and thermal spikes to the biochemical reaction systems and biomolecular systems via information technique. Low energy electron beam cause ionization, crosslinking reaction, and finally thermal conversion from their kinetic energy. These fundamental physical response of the chemical solution in nano space would promote extremely local chemical reactions which will follow temperature dependence Arrhenius equation, then would cause nonequilibrium molecular dynamics. These local reactions in nano region could induce mass transport and energy flow in the biochemical reaction systems to generate the self-organized dynamic patterns.

EXPERIMENTAL
A chemical-computer interface was demonstrated as electron beam induced chemical reaction through Si nanomembrane. Liquid water solution (ie. culture medium) was fill in a cupule with a Si window at the bottom. An electron beam was irradiated into the water solution through the Si nanomembrane window to induced nano chemical
reaction on the interface of the Si and solution. The electron beam deflection was precisely controlled by computer generated two-dimensional patterns through two-channel digital-analog converter with 16-bit resolution. The computer generated patterns were fabricated on living cells and the cell membrane had less damaged by the electron beam patterning in cultivation condition. [2] Electron beam had enough energy to generate two-dimensional chemical reaction pattern in nano space and biocompatibility. [1-2] We applied this mechanism to control the chemical reaction in the biological systems. Real-time feedback imaging system, a high speed pattern generating system, and beam scanning system were connected to the inverted electron beam lithography (I-EBL).

Figure 1 shows conceptual schematics of direct chemical-computer interface for biochemical reaction systems of biological signal processing. This concept was designed similar to virtual reality (VR) systems for psychophysics researches. The chemical reaction display was component of the real-time closed looped system of the direct chemical-computer interface, and could display chemical, mechanical, and electrical input to the nano biochemical reaction systems. And the reactions were feedback to a mathematical model. Point spread function (PSF) of the deposition process was computed by deconvolution algorithm from the input scanning pattern and SEM images of line and space. Full width at half maximum of the PSF was less than 50 nm. [3]

I-EBL induced reactions were demonstrated as soft-cutting of the living cell and stimulation in real-time process. Myoblast cells C2C12 were cultured on the Si nanomembrane to record the response to the I-EBL soft-cutting. The cells were cultured for 1-2 days in Dulbecco's modified Eagle medium (DMEM) with 15 % fetal bovine serum as supplements. Then the cells on the Si nanomembrane were set on the equipment of the I-EBL at 2.5 keV of acceleration voltage. During real-time observation using a co-axial optical microscope with 100x objective lens, the edge of a lobopodium of the myoblast was targeted to irradiate the electron beam. The physical responses of the lobopodium to applying the electron beam were recorded using scientific complementary metal oxide semiconductor camera in high speed video recording.

RESULTS AND DISCUSSION

Real-time observation of in situ patterning showed that the rectangular gradually appeared as a visible convex texture during the EB scanning. Ref [1] summarizes time series behavior of the brightness profiles for the depositing video. The increasing brightness of the deposition pattern in the video images indicated interference of reflected light at the growing thin film during the EB scanning. Also ref [1] demonstrated luminescence of the scanning spot of the electron beam during the camera recording. The optical observed luminescent spot sizes (about 300-400 nm), were related to the acceleration voltage, were greater than real full width at half maximum of PSF (about 50-100 nm) of the chemical reaction. The resolution of the display performed two-dimensional resolution beyond the diffraction limit of the conventional optical microscope.

The edge of the lobopodium of the myoblast C2C12 cell was soft-cut by using the electron beam scanning during real-time observation. The cell was cultured on the Si nanomembrane before the processing, and the process was conducted on truly living cell. Figure 2 shows video result of the response of the cell. The lobopodium of the cell detached the focal adhesion on the Si nanomembrane with very quick contracting movement of the cell. Internal stress, which was balancing by contracting actin fiber of cytoskeleton and focal adhesion force on the surface of the Si nanomembrane, was released after the electron beam induced reaction. After the EBL processing the cell maintained its motility and proliferation potential. The cell migration and dividing was confirmed more than 1 day after.
CONCLUSION

The proposed dynamic patterning for biochemical reaction systems was demonstrated with real-time nano lithograph based in the electron beam-induced chemical reaction in culturing condition. And the direct cell processing was successfully demonstrated at precisely targeted position on a truly living cell. That 50-nm resolution was enough to process cells and biochemical reaction systems with our chemical-computer interface via EB-induced chemical reactions.

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