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Production of protein microarrays for cell culture using electrostatic deposition

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Abstract

We used the electrostatic deposition (ESD) method to prepare the protein microarray for observation of the stem cell responses to pattern size, space and shapes. The ESD method allows a reduction in spot size, high efficiency of substance transfer, and high rate in fabrication as a result of ability to simultaneously deposit thousands of identical spots. Typical electrospraying conditions for the deposition of proteins were a voltage of 3.5–4.5 keV and the humidity under 40%. The patterns of masks have a variety of shapes, spaces, and hole sizes from 10 to 300 μm. Collagen is deposited on conducting polymer in a dry state, preserving the functional activity of proteins. The surface morphology of deposited spots was observed using an atomic force microscope and a scanning electron microscope. Human mesenchymal stem cells were cultured on each extracelluar protein patterned sample and cellular morphology was observed by light microscopy. Cell attachment and spreading on the surface are dependent on the deposition conditions by ESD. Protein deposition with short time is effective on the cell culture. However, collagen pattern size, space and shapes in micrometer size seemed to have little effect on cellular response in our design.

Keywords: ESD; Stencil mask; Protein microarray; Cell culture

1. Introduction

Recently, the researches of the cell response on micro/nano-structure surfaces are being in active progress in nanobiology field. Even though the size of cells is about 20 μm in general, it has been reported that cells can recognize the structural changes in nano-scale [1,2]. Adhesion, arrangement, and speed of proliferation of cell are influenced by the topography and structures on the surface in nano-scale. It means that the biological and physiological function of cells can be modulated by culturing cells on the various patterned surfaces. Especially, attentions are focused on the control of proliferation by surface control of stem sell. Furthermore, to culture cells on designated spots or on patterns is an important elementary technique in biochip, biosensor, and tissue engineering.

The representative methods of forming bio-micro/nano patterns are micro spotting [3], inkjet printing [4], microcontact printing (μCP) [5], and electrostatic deposition (ESD) using shadow mask [6]. Micro spotting and inkjet printing methods are restricted in their pattern size and pattern forming speed. Microcontact printing method has the advantages of obtaining high resolution and simultaneous patterning of multiple arrays, on the other hand, the quantity of substance transferred from PDMS stamp is limited and it is difficult to replicate patterns onto diverse surfaces. ESD method was developed to deposit protein or electrically charged nano particles on specific area of conductive substrate by electrostatic field control. Deposit quantity can be controlled by drop size, concentration of specimen and injection time. Multiple patterns can be formed simultaneously on wide area at high speed.

ESD was first introduced in the nuclear research to fabricate thin uniform radioactive sources [7]. ESD has been widely used in the various field. Very well known application of ESD is preparation of samples for mass spectrometry [8]. Other applications include the formation of metal oxide films [9], polymer film coating on the electrode and plastic substrate [10,11], DNA sample preparation [12], and protein films [13]. Recently, ESD has also been used to prepare samples of biocompatible materials to see cell behavior [14,15], DNA and protein microarrays for biochip [13,16,17], and living cells [18]. A central problem

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in the ESD of the proteins is in preserving functional properties of these molecules upon electrospray and subsequent impact of the charged molecules with a substrate surface. The results of researches show that biomolecular activities are fully preserved after spraying [16–18]. However, microarrays of proteins for cell patterning and culture have never been produced by the ESD method.

In this research, an ESD device was newly developed for micro-patterning. Protein microarrays were formed with the developed ESD and reaction of stem cells was observed according to the pattern.

2. Experiment

2.1. ESD device

Fig. 1 shows schematic diagram of the developed patterning system, ESD as well as Fig. 2 shows the picture of ESD. The ESD device incorporates a capillary holder, a guide ring, guiding gauze, Teflon shield, and sample substrate in acryl chamber. High voltage applied inside the capillary generates focused electrostatic force which ejects the charged particles in micron sized drops. Charged particles are divided sequentially by repulsion force of the charge and guided towards the mask by electrostatic field. Fine particles passed through the mask deposit on the substrate below. The substrate was rotated by motor to deposit the proteins uniformly. Since the fine particles formed with electrostatic force are too small to view with bare eyes, the visualization was achieved using a laser and a camera. The laser installed on the left scans right below the capillary tip. Laser rays diffused by particles are observed with the camera on the right. Specifications of the ESD developed in this research are listed in Table 1.

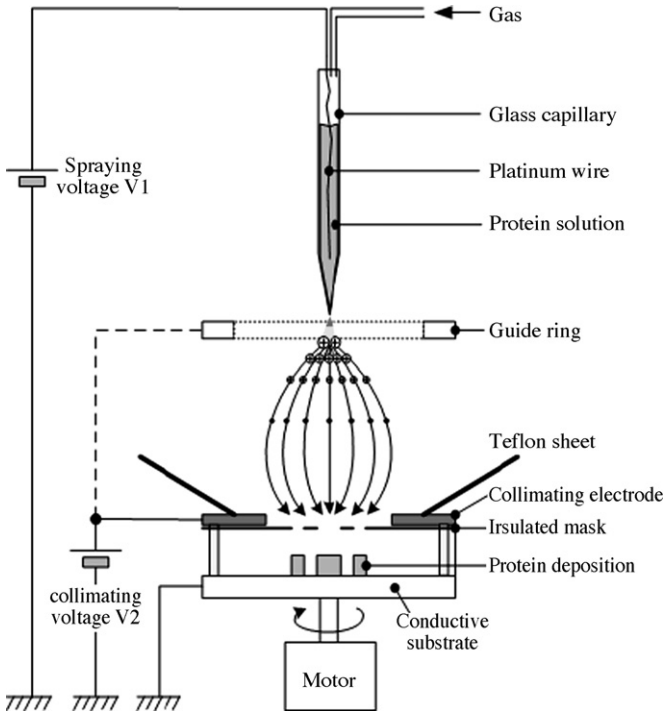

Fig. 1. The schematic diagram of the electrostatic deposition system.

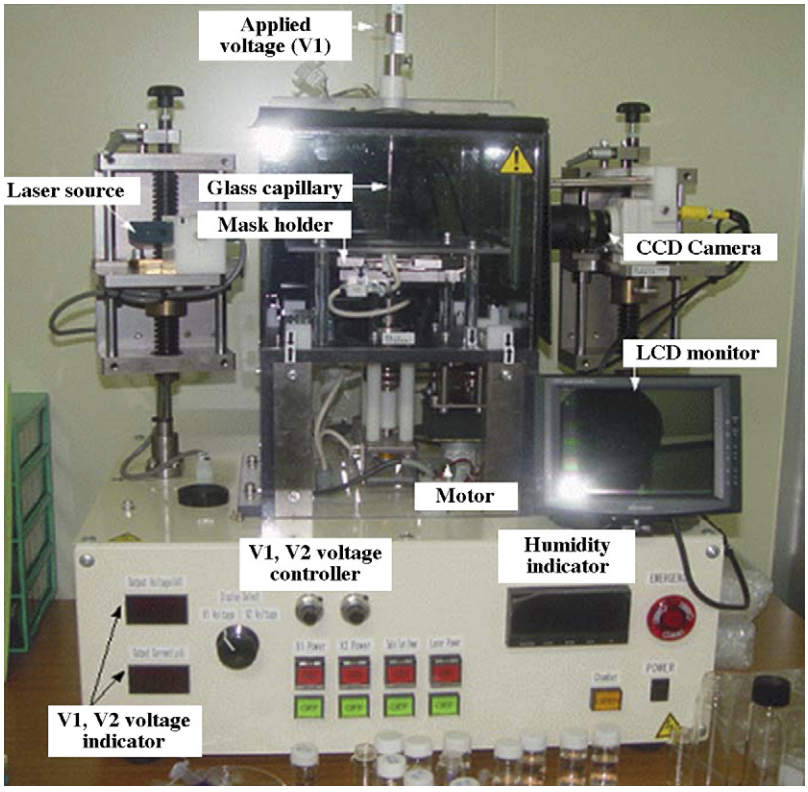

Fig. 2. The picture of developed ESD device.