PHOTO-DYNAMIC CONVERSION OF SOLID SURFACE FROM PROTEIN-PHOBIC TO PROTEIN-PHILIC BY FEMTOSECOND LASER THROUGH IN SITU MICROFABRICATION

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ABSTRACT

The regulation of proteins adsorption on solid surface is crucial to the development of devices for micro total analysis system (μTAS). This study is aiming for the dynamic surface conversion on a solid platform through laser-assisted ablation in aqueous solution. When femtosecond laser pulses (800 nm, 1 kHz, 200-500 nJ/pulse) was focused on the glass platform coated with protein-phobic polymers, the resulting ablation would reveal the bare glass surface that is protein-philic. Since the conversion would allow patterning of proteins under aqueous conditions, including physiologically buffered one, the method is suitable for pattering functional proteins with minimal damage. We have successfully applied the method in patterning extracellular matrix (ECM) proteins and culturing living cells on the patterned surface in a controlled spatiotemporal manner according to the designed pattern. We believe the method will uniquely contribute to the development of functional protein based cytology devices.

KEYWORDS

Photo-dynamic surface conversion, femtosecond laser, ECM, protein array, cell array.

INTRODUCTION

The recent growing interest in microarray devices has motivated much research in related biological applications, such as genome and proteome analysis.[1] Along the same line, living cells can also be arrayed on microfabricated cytophilic small domains on a cytophobic surface.[2] Cell adhesion is mediated by the ECM proteins e.g. collagen, laminin, fibronectin and so on. Some cells, such as neurons, require specific proteins for attaching to solid surface.[3] Accordingly, due to the complexity of protein mediated cell adhesion, controlling proteins adsorption on solid surface is essential to develop μTAS devices that are fabricated by patterning proteins and living cells. In forming patterns of proteins on solid surfaces, protein-philic surfaces must be differentiated from protein-phobic ones. A number of fabrication processes of patterns of protein-phobic and protein-philic have been developed for such purposes, including laser processing, microstamping, inkjet printing, and photolithography processing.[2] In contrast to these conventional patterning methods, our patterning approach is applicable *in situ* under aqueous condition. It is based on lithography techniques using femtosecond laser (fs laser) to convert effectively the characteristics of solid surface.[4]

In this paper, this method is further improved to convert the surface dynamically from protein-phobic to protein-philic and to replace an adsorbed protein to other ones under physiological condition. The method was successfully applied to ECM patterning, resulting enhancement of cell adhesion on designed small domains on the platform (Figure 1). All processing could be completed during a series of culturing in the medium. The *in situ* replacement of ECM has also been demonstrated under aqueous condition.

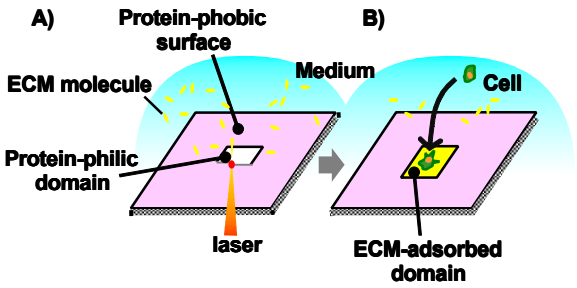

*Fig.1. Schematic illustrations of ECM patterning and cell adhesion. **A**) The surface of small domain changes from protein-phobic to protein-philic by fs laser light. **B**) ECM in the medium is adsorbed on the protein-philic domain to ensure the cell adhesion.*

EXPERIMENT

The glass substrate was covered with a copolymer of 2-methacryloyloxyethylphosphorylcholine (MPC polymer) that prevents nonspecific adsorption of proteins onto solids. To demonstrate our surface conversion, selected nanoparticles Q-dots having polyethyleneglycol (neutral), carboxyl (acidic), and amino residues (basic) on the surfaces were firstly tested with laser-irradiated MPC-polymer glass (Figure 2). The intact MPC-polymer surface did not adsorb any type of Q-dots. For comparison, the laser-irradiated surface adsorbed the amino Q-dots well, while the same surface adsorbed the neutral Q-dot slightly and hardly the carboxyl Q-dot. The laser-treated surface is now

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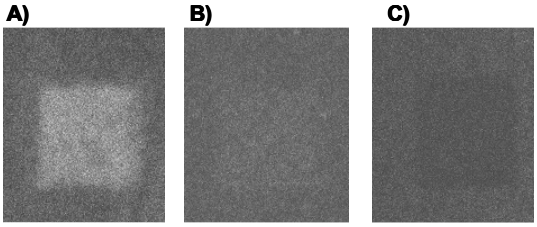

*Fig. 2. Fluorescence micrographs of fs-laser treated surface after incubated with **A**) amino, **B**) neutral, and **C**) carboxyl Q-dots. The fs laser (800 nm, 120 fs, 1 kHz, 0.5 mW) was focused onto the glass covered with MPC polymer.*

Table 1. Surface analysis

Elements	Binding energy / eV	Surface amount / mol%		
		Laser (-)	Laser (+)	Glass
C 1s	284.8	34.8	17.3	6.0
	286.2	20.5	12.2	0
	288.6	7.1	4.5	0.9
	291.0	0	0.4	0
	293.4	0	0.4	0
N 1s	402.2	2.7	2.0	0.1
O 1s	529.8	3.3	0	0
	532.1	26.4	45.3	61.6
P 2p	133.4	5.2	2.2	0
Si 2p	102.8	0	15.7	31.4

interacting with amino Q-dots that have basic charges on the surface.

The chemicals on the surface were analyzed by X-ray photoelectron spectroscopy (XPS) (Table 1). Si was not detected on the surface covered with MPC polymer while its composition on glass surface was 31.4% (molecular ratio). The Si composition was recovered to 15.7% on the laser-drawn domain. In contrast, the carbon was decreased from 62.4 to 34.8% at fs laser-drawn domains. The XPS showed 44-50% MPC polymer surface disappears. XPS reveals the laser ablates MPC-polymer to bare the grass surface. It is well known the glass surface has silanol residues being slightly negatively charged. Accordingly, the fs-laser peels the MPC-polymer to allow interaction with the surface and particles of amino Q-dot.

The method was applied to create patterns of ECM proteins on a transparent glass that was covered with MPC polymer (Figure 3). Two types of ECMs were prepared, which were collagen and laminin conjugated with Alexa Fluore 488 and 555, respectively. The fs laser-irradiated platform was then treated with fluorescence-conjugated collagen to differentiate the treated domain from the surrounding. The strong fluorescence was detected from the laser treated domain (A). Since surrounding MPC polymer area did not emit fluorescence, it was proved that the laser converted the protein-phobic to protein-philic surface. The fs laser was again focused onto the collagen-adsorbed domain and the substrate was incubated in laminin solution. The fluorescence from the laminin was emitted from the ablated area (B). The fluorescence area at laminin was clearly distinguished from collagen area. This observation indicates that our fs-laser-based dynamic surface-conversion technique can create various domains covered with a variety of ECMs. Furthermore, the domain once covered with ECM can be dynamically converted to the surface with a different ECM spatiotemporally.

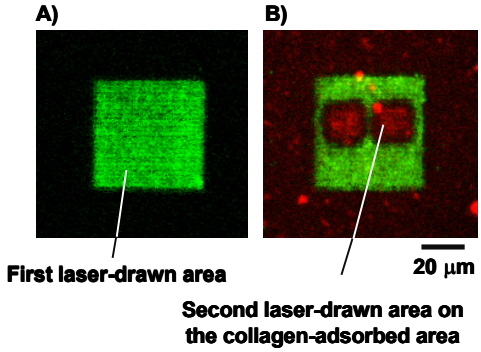

Figure 3. Multiple ECM patterning by fs laser. Green and red colours were obtained from collagen conjugated with Alexa Fluor 488 and laminin conjugated with Alexa Fluor 555, respectively. Micrographs A) and B) are taken from the plates after fs laser treated and incubated with collagen, after laser treated again on the collagen adsorbed domain and laminin treated, respectively. The fs laser (0.5 mW) was irradiated 1 μm pitches intervals to create every domain.

Finally, we have demonstrated the patterning of living cells (Figure 4). Gelatine was used to assist the cell adhesion during laser treatment. When normal human keratinocyte (NHK) was seeded on the platform, NHK adhered to the laser-drawn area (A). Another laser-drawn area was created adjacent to the former area (B). A HeLa cell adhered to the newly drawn area. The NHA adhered hardly on the laser drawn-area when the gelatine was not included in medium. Hence the results indicate our laser-assisted ECM-patterning procedure is an indispensable method for cell arraying.

CONCLUSIONS

The MPC polymer is immobilized on the solid surface by physisorption. Accordingly the fs laser ablation removes the polymer under aqueous condition. The proteins (e.g. collagen, laminin, gelatine) then attach to the ablated domains. This fs laser-based surface-conversion is applicable to many surfaces. The protein-phobic organic materials, e.g. perfluoroalkyl and alkane residues, that are chemically immobilized to solid surface can be photochemically modified to protein-philic surface by fs laser irradiation.[4,5]

The unique advantage of our method is that the protein coated surface can be converted dynamically to other protein coated surface repeatedly under physiological conditions. Applying the surface conversion, various cells have efficiently arranged on the destined area, because the receptors on cells identify the ECM molecules to

complete cell adhesion. Since it is possible to create a substrate possessing different ECMs, we will then be able to array and co-culture cells requiring different ECMs. We believe our method will contribute uniquely to the study of the many cellular processes relating to cell division, differentiation, and migration. This technique will be highly valuable to develop protein and living-cell based devices.

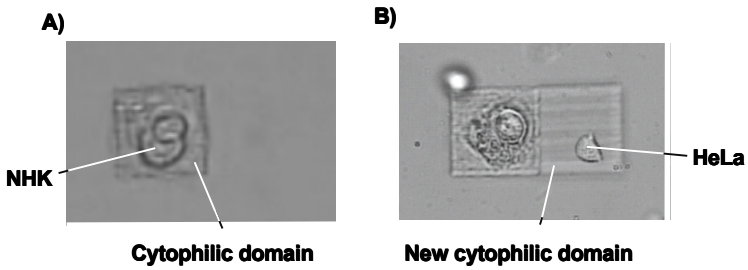

Figure 4. Arrangement of different kind of cells on a glass. Single NHA was placed on a laser treated domain (A) and next the new domain was created adjacent to NHK domain to adhere HeLa cell (B). The each cytophilic domains (50 × 50 μm) were created by applying fs laser (0.5 mW) with 2 μm pitches intervals.

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