HIGH-THROUGHPUT PROTEIN MICROARRAYS: FEATURE SIZE EFFECTS ON PRINTING ARRAYS WITH IN SITU PROTEIN SYNTHESIS

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ABSTRACT

We describe the development of a simple and versatile approach for fabricating protein microarrays using a microintaglio printing method. Unlike conventional approaches that require multiple downstream reactions to synthesize proteins off-chip followed by printing using a robotic spotter, in our approach we can simultaneously synthesize and capture functional proteins *in situ* on chip without a spotter. We successfully demonstrated the concept validity by printing green fluorescent proteins (GFPs) on a glass surface, and our results for capture spot miniaturization (100-20 μm size scales) show that the sensitivity of the protein microarray signal becomes limited as the spot size decreases.

KEYWORDS

protein microarray, cell-free protein synthesis, His-tag, PDMS, GFP

INTRODUCTION

Microarrays are helpful for high-throughput screening in the field of high-speed molecular evolution technology [1]. Protein printing methods such as the spotting method and microcontact printing method are already known [2]. In the emerging field of molecular evolution technology, it is necessary to deal with very large mutant protein libraries and therefore, a high-throughput platform is desired. For these purposes, recently we introduced a simple and robust biomolecular patterning technology called microintaglio printing (μIP) [3]. In this work, we demonstrated the direct conversion of DNA arrays into protein arrays using microintaglio printing and cell-free coupled transcription/translation system.

EXPERIMENT

A schematic of our protein microarray concept is depicted in Figure 1. For spot size scale evaluation, a polydimethylsiloxane (PDMS)-based mold with various well sizes was fabricated using a soft lithography method. The depth of each well was kept constant at 40 μm and the well diameters were 100, 90, 80, 70, 60, 50, 45, 40, 35, 30, 25, and 20 μm. For protein synthesis, a wheat-germ-based coupled transcription-translation system (Promega) was used as a cell-free system and a cDNA-encoding GFP tagged with double histidine (His), which specifically binds with Ni-nitrilotriacetic acid (Ni-NTA), was used as a template. A solution containing the cell-free system and template was mixed and applied onto the mold. The mold was then sealed with a glass substrate precoated with Ni-NTA, which is depicted in Figure 2, and incubated at 30°C for 2 h. GFPs were successfully synthesized and immobilized onto the Ni-NTA glass substrate. Following washing with phosphate buffer saline (PBS) containing 0.05% (v/v) Tween 20 (Sigma-Aldrich), fluorescence images were observed by confocal laser microscopy (Ex: 488 nm, Em: 515BP30 nm).

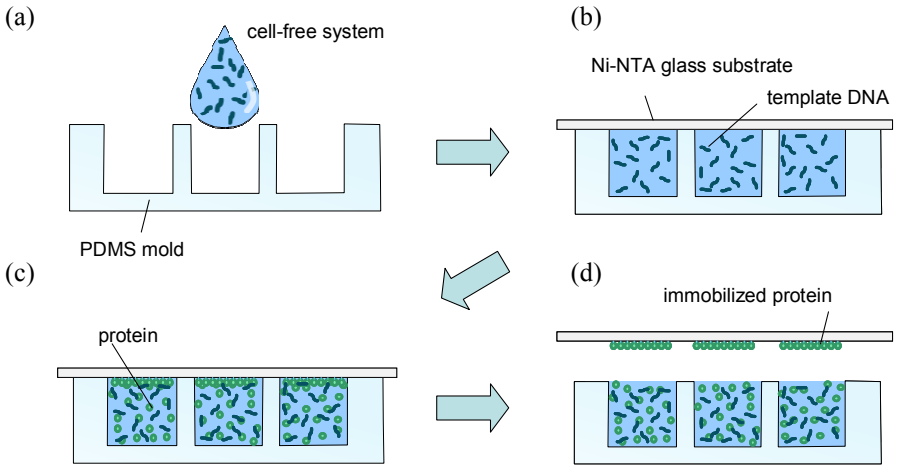

Figure 1. Development of protein microarray using microintaglio printing. (a) A mixture of cell-free system and template DNA tagged with double histidine is dropped on a PDMS mold. (b) The mold is sealed with a Ni-NTA glass substrate. (c) After incubation at 30°C for 2 h, proteins were synthesized and immobilized onto the Ni-NTA glass substrate. (d) Proteins are captured in situ on the Ni-NTA glass substrate.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

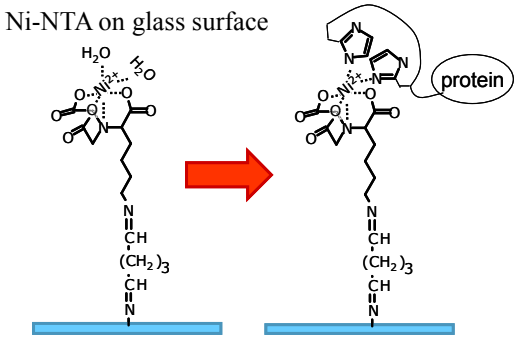

Figure 2. To immobilize His-tagged protein, glass surface was coated with Ni-nitrilotriacetic acid (Ni-NTA). Histidines bind to Ni-NTA with covalent coordinate bond.

RESULTS AND DISCUSSION

Figure 3 shows fluorescence images of immobilized GFPs obtained using confocal laser microscopy. We observed a size effect that the spot intensity of immobilized GFP decreases with the diameter of the wells. One of possibilities is that the size effect was caused by free histidines which competitively bind to Ni-NTA. To evaluate effect of competitive binding of free histidines to Ni-NTA, we immobilized GFPs on Ni-NTA glass substrate using microintaglio printing at three amino acid concentrations which were 20 μM, 100 μM, and 150 μM. The results are shown in Figure 4. This decrease in spot intensity was also observed to limit the reaction when the concentration of amino acids was decreased. Therefore the size effect was not caused by the competitive binding. Moreover, as shown in Figure 5, a size effect was also observed for the concentration of GFPs synthesized within each well. In contrast, size effect was not observed for microintaglio printing with off-chip protein synthesis as shown in Figure 6. We believe that the area density of GFPs decreased by the reduction of the synthesis concentration. To explain this, it is necessary to consider the adsorption of the nucleic acids and proteins on the surface of the well. Since the ratio of the surface area of PDMS to the reaction volume increases with the diameter of the well, relative adsorption on the surface also increases.

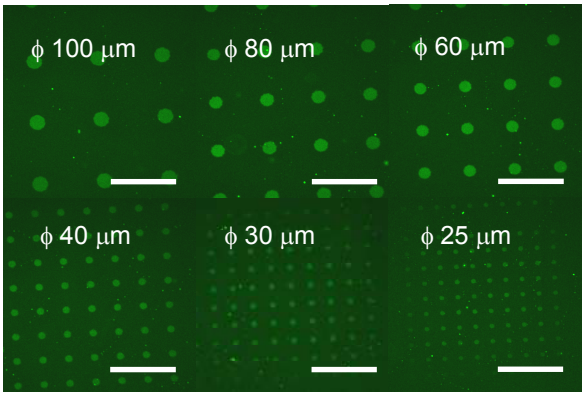

Figure 3. Fluorescence images of immobilized GFPs obtained using confocal laser microscopy (Ex: 488 nm, Em: 515 BP30 nm) All scale bars are 400 μm.

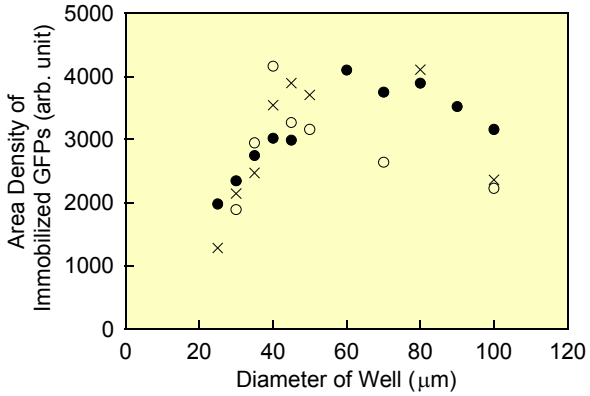

Figure 4. Relation between diameter of the well and relative area density of immobilized GFPs synthesized at amino acid concentrations of 20 μM (filled circles), 100 μM (open circles), and 150 μM (crosses) Regardless of amino acid concentration the spot intensity of immobilized GFP decreased with the diameter of the wells.

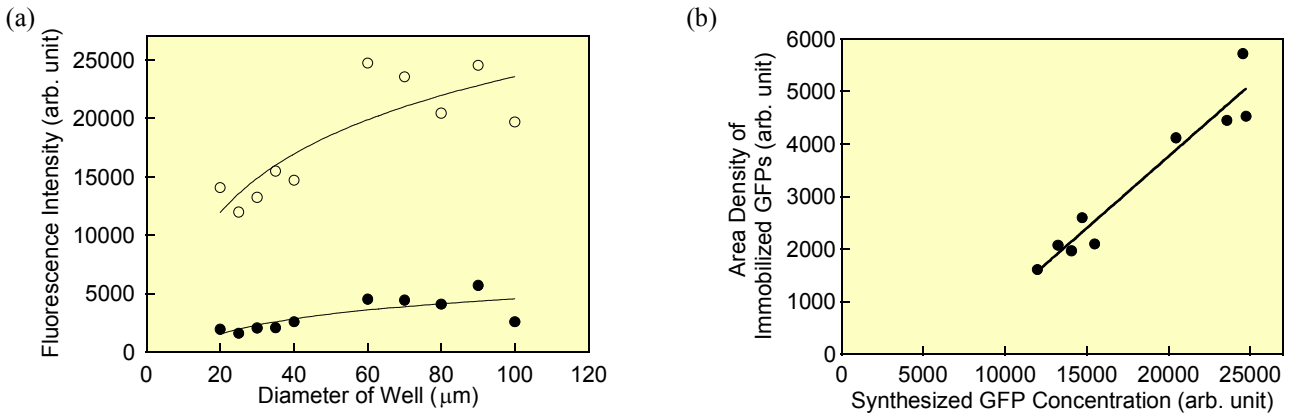

Figure 5. (a) Dependence of synthesized GFP concentration on diameter of the well. Filled circles show the relative synthesized GFP concentration. Open circles represent the relative area density of immobilized GFPs. a size effect was also observed for not only the area density of immobilized GFPs but also the concentration of GFPs synthesized within each well. (b) Relation between density and concentration of GFPs. Area density of immobilized GFPs was correlated with the synthesized GFP concentration.

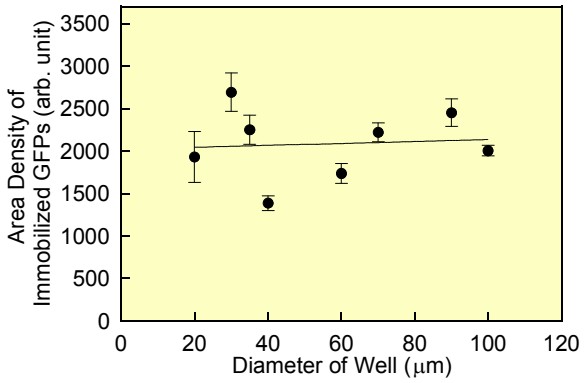

Figure 6. Plot of area density of GFPs immobilized using microintaglio printing with off-chip protein synthesis vs. diameter of the well. Size effect was not observed.

CONCLUSION

We successfully demonstrated validity of microintaglio printing by printing GFPs on a glass surface. Our results for printing spot miniaturization (100-20 μm size scales) show that the spot intensity of immobilized GFP decreased with the diameter of the wells. The area density of GFPs decreases as the synthesis concentration reduces.

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