Micropatterning of Different Kinds of Biomaterials As a Platform of a Molecular Communication System

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***Abstract*—We aimed to create a method in micropatterning of different kinds of biomaterials as a platform of a molecular communication system onto a single substrate. This paper proposes a multiple poly(para-xylylene) (parylene) simultaneous peel-off process and demonstrates that different kinds of proteins and DNAs were successfully microarrayed onto the single substrate. Further, the functionalities and the contamination-free nature of these microarrayed biomaterials were maintained throughout the micropatterning process. Our results contribute to the development of microarrayed senders and receivers such as DNA-tagged vesicles and/or biological cells in molecular communication, and will help to investigate and visualize the overall of molecular communication processes.**

Keywords-molecular communication; microarray; parylene peel-off process; lyophilization; DNA; protein

I. INTRODUCTION

Molecular communication [1]-[3] is inspired by the biological communication mechanisms (e.g., cell-cell communication using hormones) [4],[5] and artificially creates a biochemically-engineered communication system in which communication processes are controllable. Molecular communication uses molecules (i.e., chemical signals) as an information medium and allows biologically- and artificially-created nano- or cell-scale entities (e.g., living cells and biohybrid devices) to communicate over a short distance. It is a new communication paradigm and is different from the traditional communication paradigm that uses electromagnetic waves (i.e., electronic and optical signals) as an information medium. Molecular communication has received increasing attention as an emerging interdisciplinary research area, which spans nanotechnology, biotechnology, and communication engineering [6]-[8].

In molecular communication, a sender encodes information onto molecules (called information molecules) and emits the information molecules to the propagation environment. A propagation system transports the emitted information molecules to a receiver. The receiver, upon receiving the transported information molecules, reacts biochemically to the received information molecules (this biochemical reaction represents decoding of the information). We have successfully constructed some model molecular communication components such as senders/receivers using lipid vesicles [9]-[11] and a propagation system using molecular motors [12]-[14]. In these previous works, however, overall of the molecular communication processes have not been demonstrated because sender/receiver vesicles have diffused in the aqueous propagation environment, or either senders or receivers have only just microarrayed. To investigate and visualize the overall of the molecular communication processes, it will be helpful to provide a method in micropatterning of different kinds of biomaterials such as proteins and DNAs to tether both of the senders and receivers onto a single substrate.

Here, we propose a multiple poly(para-xylylene) (parylene) simultaneous peel-off process to perform the micropatterning of different kinds of biomaterials as a platform of a molecular communication system (Fig. 1). Parylene is a kind of a transparent resin film, and has been received increasing attention because (1) it can be used as a stencil to produce microarrays on a large area with high resolution using the standard photolithography, (2) it can be easily peeled off from a vapor-deposited substrate, and (3) it is a biocompatible material [15]. In the proposed process, the first layer of a parylene film is used as a stencil to spot Sample A, and the spotted samples are lyophilized. Then the second layer of a parylene film is vapor-deposited onto the lyophilized substrate, and is used as a stencil to spot Sample B. Finally, the first and the second layers of parylene films are peeled off simultaneously, and the micropatterned Sample A and B are obtained on a single substrate. Unlike the existing method that uses an inkjet printing with a single parylene peel-off process [16], our proposed process does not require any spotting machines. In addition, unlike the existing method that uses a multiple parylene sequential peel-off process [17], our proposed process is contamination-free between samples. This paper is the first to demonstrate the micropatterning of different kinds of biomaterials such as proteins and DNAs onto a single substrate using the multiple parylene simultaneous peel-off process. Our results contribute to the development of microarrayed senders and receivers such as DNA-tagged vesicles and/or biological cells in molecular communication.

II. PROPOSED MICROPATTERNING PROCESS

To perform the micropatterning of different kinds of biomaterials as a platform of a molecular communication

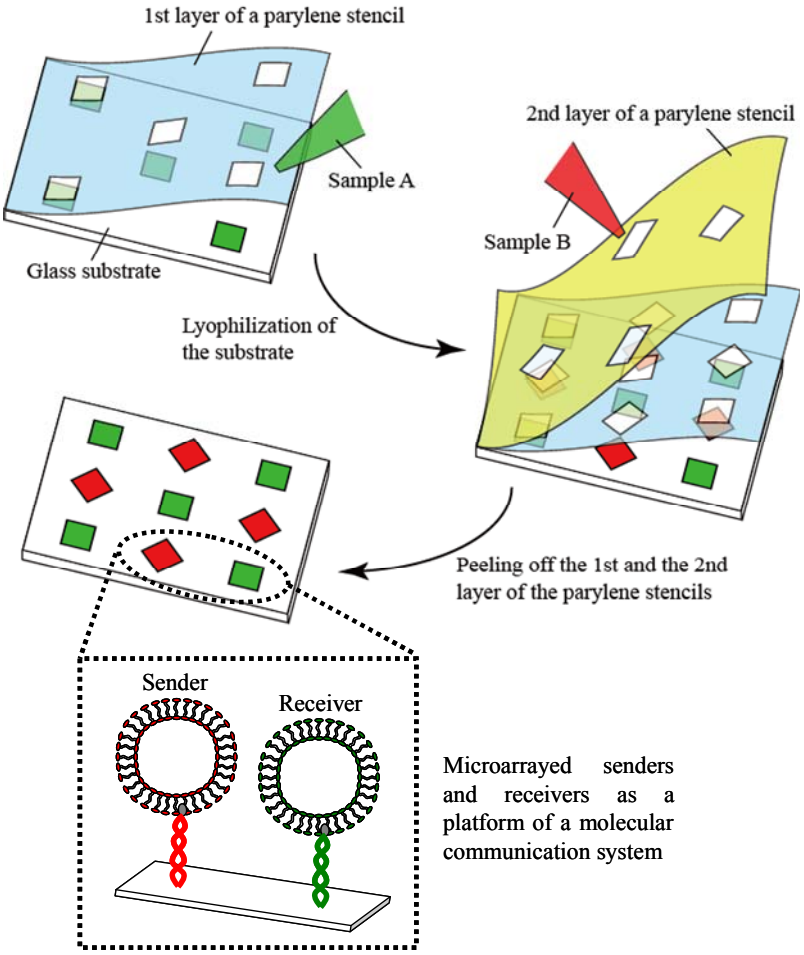

Figure 1. Conceptual diagram of our proposal.

system, we propose a multiple parylene simultaneous peel-off process (Fig. 2).

In the proposed process, the first layer of a thin parylene film (e.g., 1 μm thick) is vapor-deposited onto a glass substrate (Fig. 2 (a)). After the spin-coating of a photoresist such as S1818 (Shipley Far East Ltd.) onto the parylene-deposited substrate (Fig. 2 (b)), UV light is irradiated through a micropatterned glass photomask for the first layer (Fig. 2 (c)). The exposed photoresist is developed using the NMD-3 developer (Tokyo Ohka Kogyo Co., Ltd.) (Fig. 2 (d)), and then the vapor-deposited parylene film is dry etched by O_2 plasma exposure (Fig. 2 (e)). The remaining photoresist is removed and washed away using the acetone and isopropyl alcohol (IPA) (Fig. 2 (f)). A flow cell is configured on the micropatterned parylene film, and the glass substrate is coated with a solution containing Sample A (Fig. 2 (g)). After the solution is exchanged to the distilled water, the whole substrate with the flow cell is lyophilized using the liquid nitrogen and a vacuum freeze dryer (Fig. 2 (h)).

The flow cell is removed from the lyophilized substrate, and the second layer of a thin parylene film (e.g., 1 μm thick) is vapor-deposited onto the first layer of the micropatterned parylene film and the lyophilized Sample A (Fig. 2 (i)). After the spin-coating of a photoresist such as S1818 onto the parylene-deposited substrate (Fig. 2 (j)), UV light is irradiated through a micropatterned glass photomask for the second layer (Fig. 2 (k)). The exposed photoresist is developed using the NMD-3 developer (Fig. 2 (l)), and then the first and the second layers of parylene films are dry etched by O_2 plasma exposure (Fig. 2 (m)). The remaining photoresist is removed and washed away using the acetone and IPA (Fig. 2 (n)). A flow cell is configured on the micropatterned parylene film, and the glass substrate is coated with a solution containing Sample B (Fig. 2 (o)). Note that the lyophilized Sample A is covered with the second layer of the parylene film, and thus the poured Sample B is not mixed with Sample A at all. Lastly, the first and the second layers of parylene films are peeled off simultaneously, and the micropatterned Sample A and B are obtained on a single substrate (Fig. 2 (p)). The lyophilized Sample A is refused and is ready for use by pouring an appropriate buffer solution (Fig. 2 (q)).

III. Experimental Results and Discussion

We first tried to confirm the feasibility of our proposed micropatterning process by using fluorescence microscopy. We used 5 μM biotinylated, fluorescein isothiocyanate (FITC)-labeled 23-base single-stranded DNAs (ssDNAs) as Sample A,

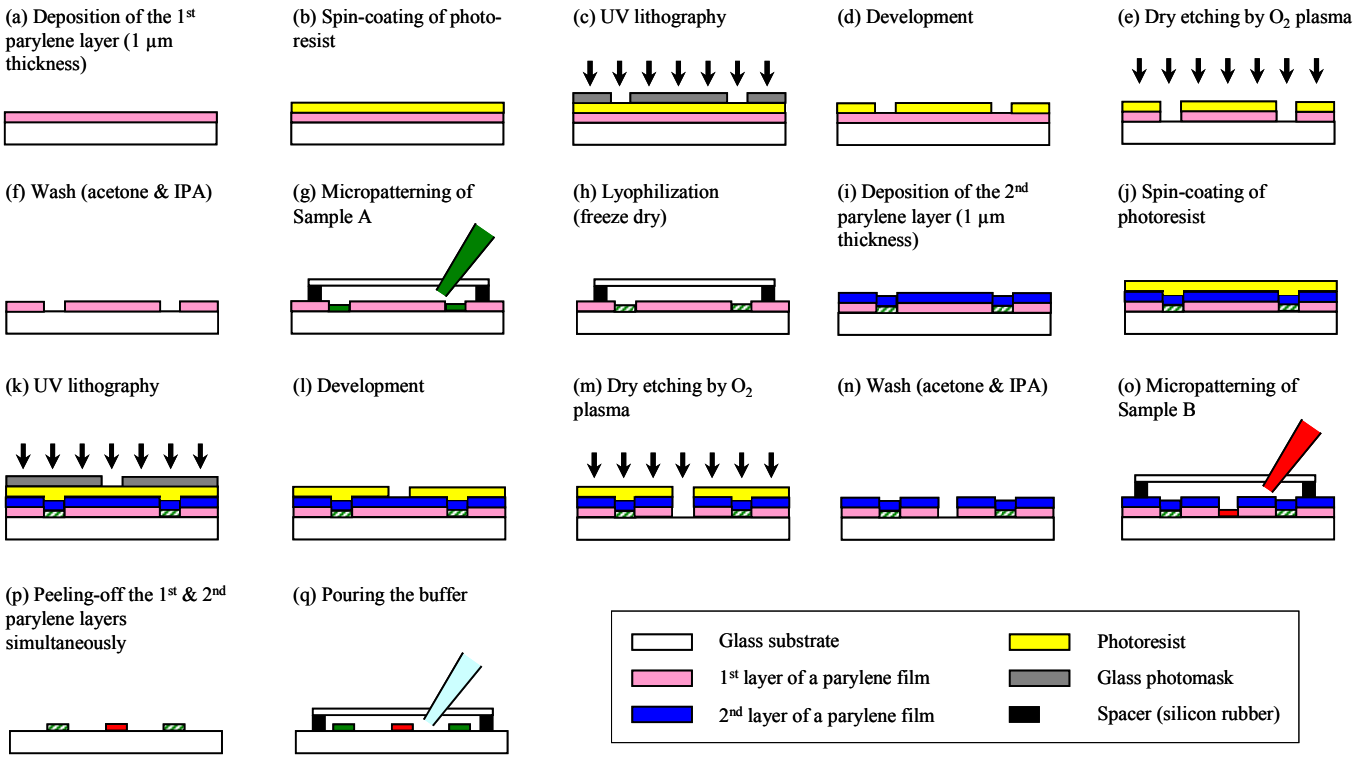

Figure 2. Schematic diagram of our proposed micropatterning process.

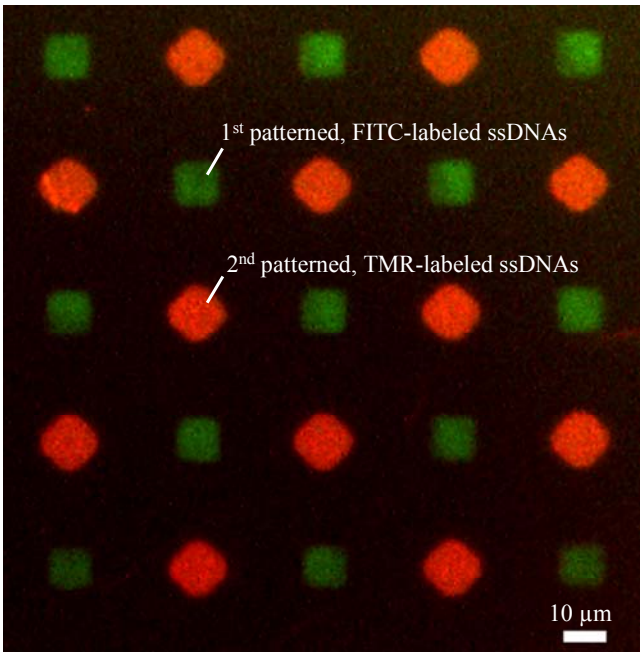

Figure 3. Micropatterned two kinds of ssDNAs onto a single substrate.

and the ssDNAs were tethered through biotin-avidin bindings where 4 mg/mL biotinylated bovine serum albumin (bBSA) and 1 mg/mL streptavidin were adsorbed onto the glass substrate. Similarly, we used 5 μM biotinylated, tetramethylrhodamine (TMR)-labeled 23-base single-stranded DNAs (ssDNAs) as Sample B, and the ssDNAs were tethered

TABLE I. THE ssDNA BASE SEQUENCES USED IN THE EXPERIMENTS

	ssDNA base sequences (5' to 3')	Modifications	
		5'	3'
A	TTCGCTGATTGTAGTGTTGCACA	Biotin	FITC
B	CATCTGAACGAGTAAGGACCCCA	Biotin	TMR or FITC
$\bar{A}$	TGTGCAACACTACAATCAGCGAA	Cholesterol	TMR

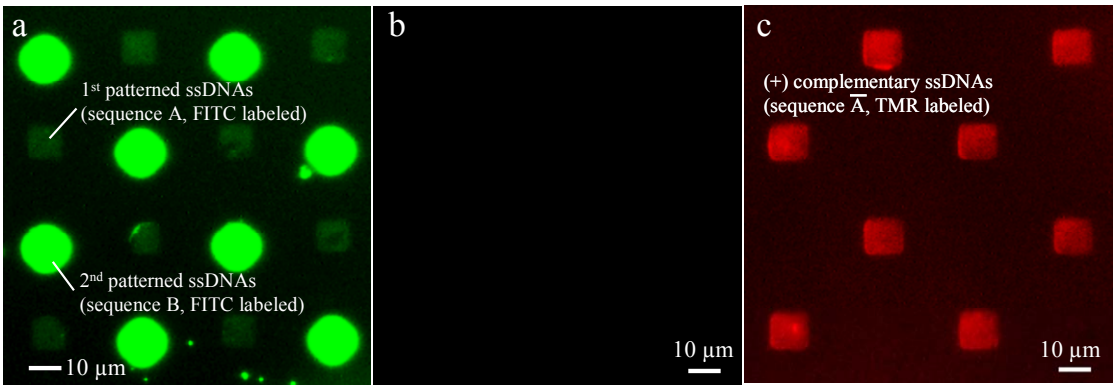

Figure 4. Micropatterned ssDNAs and their DNA hybridization capabilities.

through biotin-avidin bindings where 4 mg/mL bBSA and 1 mg/mL streptavidin were adsorbed onto the glass substrate. Fig. 3 shows that the two kinds of protein-anchored ssDNAs were successfully microarrayed on a single substrate using the proposed multiple parylene simultaneous peel-off process. As shown in this figure, green- or red-colored ssDNAs were not overlaid at all, and it proved that our proposed process is contamination-free. Although we employed dry etching by O_2 plasma exposure this time, the patterning resolution of a parylene film can be downscaled to nanometers if we employ electron beam lithography. Further, it is easy to imagine that if we stack three or more parylene films by repeating the proposed micropatterning process depicted in Fig. 2, three or more kinds of biomaterials can be microarrayed.

To confirm the functionalities of the observed microarrays, we examined DNA hybridization capabilities of the lyophilized ssDNAs. The ssDNA base sequences used in the experiments are summarized in Table I, and were selected from a set of orthogonal base sequences, designed according to theoretical and experimental considerations [18],[19], to minimize mismatched hybridization. We used 5 μM biotinylated, FITC-labeled 23-base ssDNAs with sequence A and sequence B as Sample A and Sample B, respectively, and the ssDNAs were tethered through biotin-avidin bindings as described above. Figs. 4a and 4b show fluorescence micrographs of the substrate observed through filters of FITC and TMR, respectively. We confirmed that we can see microarrays of Sample A and Sample B only through the filter of FITC. Then we poured 5 μM cholesterol-modified, TMR-labeled 23-base ssDNAs with sequence $\bar{A}$ that are complementary to those of Sample A with sequence A, and observed the substrate through the filter of TMR. Fig. 4c shows that the poured ssDNAs with sequence $\bar{A}$ were selectively hybridized with the microarrayed Sample A with sequence A, and were not hybridized with Sample B with sequence B. These results indicate that DNA hybridization capabilities are maintained even after the lyophilization and the dry etching process. Considering that the lyophilized ssDNAs were tethered through proteins (i.e., bBSA and streptavidin), it implies that the functionalities of the lyophilized proteins are also maintained throughout the proposed micropatterning process.

IV. ENVISAGED MOLECULAR COMMUNICATION SYSTEM

Based on the present results, it will be possible to devise a molecular communication system with microarrayed senders and receivers onto a single substrate. In molecular communication, lipid vesicles and biological cells are typically used as model senders and receivers [3]. Although they will not endure the lyophilization process, we can biochemically modify their surfaces with specific cross-linkers such as ssDNAs [11],[14],[20], biotins [21], and antibodies [22]. These facts imply that senders and receivers can be microarrayed through the DNA hybridization, biotin-avidin bindings, and antigen-antibody bindings onto a single substrate where complimentary ssDNAs and proteins are microarrayed using the proposed process. By combining the microarrayed senders and receivers with a propagation system using molecular motors [12]-[14], we can construct a model molecular communication system that is suitable for the investigation and

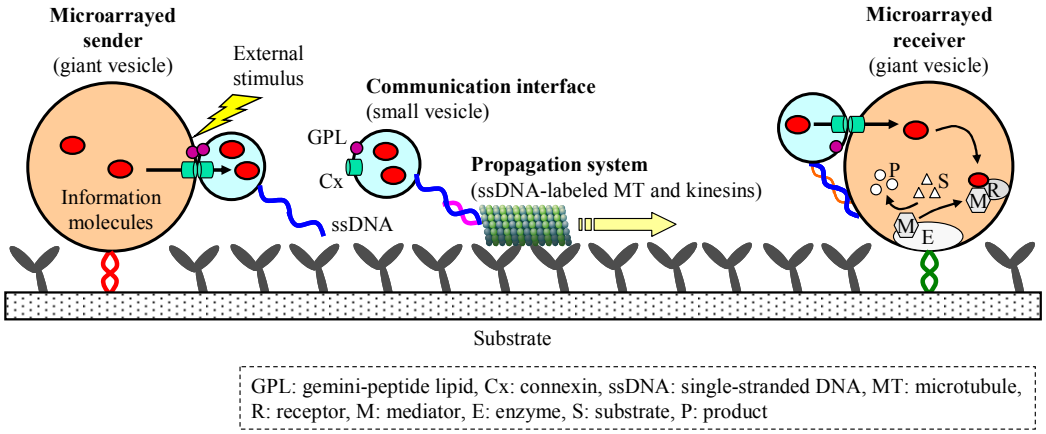

Figure 5. Model molecular communication system.

visualization of overall of the molecular communication processes (Fig. 5).

A giant vesicle (i.e., a sender) and a small vesicle (i.e., a molecular communication interface) have the same types of photo-responsive gemini-peptide lipids (GPLs) [3],[9] and channel-forming connexins [3] in their lipid bilayer membranes, and information molecules are stored in the inner aqueous phase of the sender vesicle. When UV light irradiation is applied as an external stimulus, the sender vesicle and the small vesicle assemble stably, and a gap junction channel is formed between the sender vesicle and the small vesicle. Next, information molecules are transferred from the sender vesicle to the small vesicle according to the molecular concentration gradient.

When visible light irradiation is applied as a trigger of sending, the small vesicle dissociates from the sender and information molecules are encapsulated in the inner aqueous phase of the small vesicle. The small vesicle also has ssDNAs that are complementary to those of a microtubule (MT), and it is loaded onto the gliding MT through DNA hybridization. The loaded, small vesicle is transported by MT motility on kinesin molecular motors [12]-[14] from the sender vesicle to a giant receiver vesicle, which has ssDNAs complementary to those of the small vesicle and channel-forming connexins.

At the receiver vesicle, the transported small vesicle is unloaded from the gliding MT through DNA hybridization (strand exchange), and connexins in the membranes of the small vesicle and the receiver vesicle physically and spontaneously come into contact with each other. Then a gap junction channel is formed between the small vesicle and the receiver vesicle and information molecules are transferred from the small vesicle to the receiver vesicle according to the molecular concentration gradient. The decapsulated information molecules are captured by GPLs as artificial receptors and then metal ions (as mediators) bind to the information molecule-receptor complex. Consequently, enzymatic activities are switched on, and signal amplification results in the biochemical reaction at the receiver.

This model system can be operated without using external power or control as long as an external stimulus, such as a trigger of sending, is applied to the system. These features will help to create highly miniaturized and scalable systems.

V. CONCLUSIONS

We have demonstrated the feasibility of our proposed multiple parylene simultaneous peel-off process. In the present micropatterning process, different kinds of biomaterials such as proteins and DNAs were successfully microarrayed onto a single substrate, and the functionalities and the contamination-free nature of these microarrayed biomaterials were maintained throughout the process. Our next challenges will include the investigation and visualization of overall of the molecular communication processes using the microarrayed senders and receivers.

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