Separation capability of proteins using microfluidic system with dendrimer modified surface

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Microscale and nanoscale chip are adaptable to the future of microreactors and small sensors. Interactions between proteins and biomaterials surfaces correlate with many important phenomena in biological systems. In this research, we investigated the separation capability of proteins using microfluidic system, involving modified surface with poly(amidoamine) (PAMAM) dendrimer and poly(2-methacryloyloxyethyl phosphorylcholine(MPC)-co-n-butyl methacrylate(BMA)) (PMB30W). The PAMAM dendrimer and PMB30W provided a facile way to inhibit non-specific protein adsorption, and influenced flow velocities of proteins. We believe the microfluidic device with dendrimer coated surface plays important role in the development of useful materials for biomolecular separation.

Key words: Microchip, Dendrimer, Protein separation, Bioinert surface

1. INTRODUCTION

The concepts of micro total analysis systems (μTAS) and micro electro mechanical system (MEMS) are necessary for the development of analytical instruments with miniaturized [1]. The tiny reactor or sensor systems with micro- or nano-scale have a possibility to accumulate, which are adaptable to the future materials in our everyday life. The nanofluidic device is an attractive tool for biomolecular separation, sample enrichment, and even single molecule analysis, which represents higher performance for chemical engineering [2, 3]. Poly(dimethylsiloxane) (PDMS) is now popular material for the fabrication of microfluidic devices such as microchannel andvalves because of its low cost, optical property and the facility of formation and processing by soft lithography [4, 5]. The main advantages of applying micro- or nano-fluidics to these assays are high throughput, short analysis time, small volume and high sensitivity, which are based on the decrease of sample requirements and the unique and special flow dynamics of nano- or micro-channels [6, 7]. In addition, the design of channel regulate the fluidics; for example, the incorporation of microbeads in nanochannel and the figuration of micropillar as solid supports can enhance the performance of microfluidic devices in the point of solute separation and extraction [8, 9]. However, there were few reports about separation and extraction processes using the microchannel with the surface coating via molecular interaction.

Interactions between proteins and biomaterials surfaces correlate with many important phenomena in biological systems, and have been used to develop various artificial biomaterials and applications [10]. Each interaction with a certain biomaterials is unique to biomolecule, and is expected to be taken advantage of the separation and extraction by the usage of the microfluidic devices. It is necessary for the biomolecule separation to have the regulation properties of the protein adsorption in the microchannels. Therefore, the combination of bioinert surface with weak interaction has been focused on, and has been reported the system involves surface coating with hydrophilic polymer brushes [11]. In fact, since the hydrophobicity of PDMS often leads to non-specific adsorption of analytes such as proteins, resulting in debased analysis performance due to a lower signal-to-noise ratio, the hydrophilic treatment of PDMS has achieved by the modification of the copolymer of 2-methacryloyloxyethyl phosphorylcholine (MPC) and n-butyl methacrylate (BMA) (PMB30W) [12] (Fig. 1). However, there was few capabilities of protein separation in this microfluidic system.

Poly(amidoamine) (PAMAM) dendrimers have been known to have well-defined rigid structures, appropriate size of the building block and a number of NH2 terminals [13]. The surface with high generation-PAMAM dendrimer brush represented bioinertness due to the high density of terminals [14], and is expected to exhibit this unique properties in micro- or nano-scale. Furthermore, the surface was also expected to have superior functionality for separation and extraction of solute biomolecule because of
representation of multivalent terminal ligands of polycations in nanoscale.

![Molecular structures of PMB30W and G4 PAMAM dendrimer.](image)

**Fig. 1** Molecular structures of PMB30W and G4 PAMAM dendrimer. into Au with the dendrimer modified.

In this research, we ambitiously projected the microfluidic system which the surface involving PAMAM dendrimer stuck to PDMS stamp, and investigated the separation capability of proteins such as bovine serum albumin (BSA) and lysozyme. The characteristics of dendrimer interface could exhibit a specific effect on µTAS system.

2. EXPERIMENTAL

2.1. Materials

The following reagents were used as received: G4 PAMAM dendrimer (ethylenediamine core), bovine serum albumin (BSA) and lysozyme (Sigma-Aldrich, MO, USA), reagents of fluorescent label (DyLight594 and DyLight488 Antibody Labeling Kit) (Thermo Scientific, IL, USA), SU-8 2025 (MicroChem Corp., MA, USA), Developer for SU-8 (Nippon Kayaku Co., Ltd., Tokyo, Japan), OFPR 800 and developer (NMD-3, 2.38%) for OFPR 800 (Tokyo Ohka Kogyo Co., Ltd., Kanagawa, Japan), and PDMS and cross-linker (Silpot 184 w/c, Toray - Dow Corning Co., Tokyo, Japan).

2.2. Microchip fabrication

The fabrication process of microchip is shown in Fig. 2. The microchip was composed of two parts, that is, the PDMS stamp and Au patterned glass substrate as microchannel. Slide glass (Matsunami Glass Ind., Ltd., Osaka, Japan) was washed with ethanol via ultrasonic cleaning process, and then was exposed to oxygen plasma just before use (300 W, 10 min) (Plasma Reactor PR500, Yamato Scientific Co., Ltd., Tokyo, Japan).

After sputtering of TiO2, the slide glass was spincoated with SU-8 2025 (negative resist) and was prebaked at high temperature [15]. The mold with orderly patterned was fabricated by means of photolithography, after the photoresist on glass slide was exposed to UV irradiation via photomask and then soluble resist was removed by photographic developer. Subsequently, the 10/1(w/w) mixed solution of PDMS/cross-linker was poured on the mold and degassed for 3 h at room temperature. Then, the curing reaction of PDMS was carried out at 60 °C for 6 h (Forced Convection Drying Oven D0-450FA, AS ONE, Osaka, Japan). After removing the PDMS stamp from the mold, the inlet/outlet holes for sample or buffer solution were processed in PDMS stamp by drilling.

Au patterning on slide glass was achieved by photolithography and sputtering. The slide glass was spincoated with OFPR 800 (positive resist) and was prebaked at high temperature. After UV irradiation, the soluble resist was removed by developer. Subsequently, Au patterning was obtained by using liftoff technique for removing unwanted Au with acetone after Au sputtering onto the positive resist coated glass slide.

Au patterning on slide glass was washed by immersion into a piranha solution (concentrated H2SO4:H2O2 = 3:1) and oxygen plasma treatment (85 W, 10 sec). Then, PDMS stamp was stuck on the Au patterned slide glass, and the microchip with Au microchannel was fabricated. The depth of microchannel was 100 µm, and the width was 200 µm and 250 µm for sample channel and analysis channel, respectively.

2.3. Surface-modification processes with dendrimer onto Au and PMB30W onto PDMS

<table>
<thead>
<tr>
<th>Negative resist</th>
<th>Positive resist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation of UV</td>
<td>Development, Rinse</td>
</tr>
<tr>
<td>Curing of PDMS</td>
<td>Sputtering of Au</td>
</tr>
<tr>
<td>Removing</td>
<td>Sticking</td>
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**Fig. 2** Schematic illustration of the microchip fabrication processes.

Table II. Each arrival time of maximum fluorescence intensity at observation point.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fluorescent molecule</th>
<th>Excitation wavelength</th>
<th>Emission wavelength</th>
<th>Arrival time (sec)</th>
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<tbody>
<tr>
<td>BSA</td>
<td>DyLight 488</td>
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Microchannel was filled with 0.10 mM aqueous solution of PAMAM dendrimers, covering inlet/outlet holes by using parafilm to prevent evaporation for 12 h. After incubation, the microchannel was washed with ultra-pure water, and the PAMAM dendrimer was modified on Au. Next, 1 w% of PMB30W aqueous solution was pipetted into the microchannel. After incubation for 1 h, the microchannel was washed with ultra-pure water, and PDMS was coated with PMB30W.

2.4. Protocol of microchip protein separation.
After the sample load channel was filled with protein sample solution (0.1 mg/mL) using micro syringe pump (ESP-32, Eicom Corp., Kyoto, Japan), PBS solution was injected into the analysis channel at 1.2 mL/h using two-type micro syringe pump (Model 100 Series Syringe Pump, KD Scientific, Inc., MA, USA), sweeping the sample solution away (Fig. 3). Flow image of sample was observed by fluorescence microscope (Axioskop 2 plus, Carl Zeiss Inc., Oberkochen, Germany) at 2.4 mm passed on Au sputtering surface and analyzed using VH Analyzer (version 2.60, KEYENCE, Osaka, Japan).

3. RESULTS AND DISCUSSION
Selective modification on Au was confirmed in advance of the fabrication of microchannel. The surface modified dendrimer on Au was monitored by contact angle goniometry and X-ray photoelectron spectroscopy (XPS) (AXIS-Hsi, Shimadzu/Kratos, Kyoto, Japan). Water contact angle of the dendrimer modified decreased into 49.7° from 83.8° of bare surface on Au. XPS spectra also indicated the dendrimer immobilization on Au because of the increase of N1s peak (Fig. 4 (a)). The pre-immune serum was monitored by XPS after the exposure to PMB30W solution (Fig. 4 (b)). XPS spectra showed that PMB30W hardly adsorbed on Au surface and was modified only PDMS surface of flow channel via modification process [12].

Subsequently, the microchip fabrication process was developed.

The patterned master of SU-8 had high durability as a mold because of TiO2 sputtering. High adhesive strength between PDMS stamp and Au patterned slide glass was achieved by removing grime on each surface. The modification of PDMS with PMB30W largely blocked non-specific protein adsorptions in the micro channel, comparing to the bare PDMS surface.

Performance of microfluidic systems is often improved by the means of sample injection, and a channel interconnects with minimal dead volumes also decreases the dispersion. In this research, however, it was difficult that sample injection volume was adjusted by syringe pump and injector, because of a small amount of sample and the dispersion. Therefore, we overcame the problem by adjusting sample volume using flow channel geometry with cruciform type (Fig. 3).

The protein separation of microchannel was evaluated by recording arrival times of protein flow at the observation point with two fluorescent labeled proteins (lysozyme and BSA) (Table I). After the sample loading into the sample channels, PBS buffer solution was flowed into the analysis channel at the flow rate of 1.2 mL/h (Fig. 3). The distance to observation point from start point was 2.4 mm, and the arrival time of maximum fluorescence intensity was observed, changing both kinds of protein and the surfaces. Arrival times of lysozyme and BSA were 1.69 sec and 1.95 sec in the bare Au microchip, respectively (Table II, Fig. 5). The difference was suggested that protein flow was affected by non-specific adsorption of protein on bare Au surface, which highly correlated with different properties of lysozyme (pI=10.9, 14 kDa) and BSA (pI=4.8, 69 kDa).

On the other hand, arrival time in the dendrimer modified microchip was same 1.04 sec using both lysozyme and BSA (Table II).

Table I. Fluorescent labeling molecule and its excitation and emission wavelengths of each protein.

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<td>1.04 [0.22]a</td>
</tr>
<tr>
<td>Control surface</td>
<td>1.69 [0.55]a</td>
</tr>
<tr>
<td>Dendrimer surface</td>
<td>1.95 [0.39]a</td>
</tr>
</tbody>
</table>

*a Half width of maximum fluorescent intensity
4. CONCLUSION

We have demonstrated the fabrication of microchip involving the microchannel with the macromolecules modified. The interface of microchannel was composed of both PMB30W covering PDMS and the dendrimer on Au, providing the unique functionalities such as the inhibition activity of non-specific protein adsorption and the shortening of protein flow. The separation capability of the dendrimer microchannel was some elucidated possibility, but perfect design was not developed. We believe that the dendrimer interface in microchannel functions as the separating materials, taking advantage of unique properties of the dendrimer.

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