DEVELOPMENT OF POLYMER-MODIFICATION METHOD FOR CREATION OF FUNCTIONAL EXTENDED NANOSPACE

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ABSTRACT

We here report a development of polymer-modification method utilizing surface-initiated atom transfer radical polymerization (SI-ATRP) for creation of functional space in a few hundreds nanometer-sized channel (so-called extended nanospace). The method which integrates bottom-up polymer-modification method with top-down fabrication technology allows us to create an extremely small-sized multi-functional space. Availability confirmation of this SI-ATRP method was first performed on microspace, and then we suggested unique function of polymer-modified extended nanospace for novel protein separation device.

KEYWORDS: Extended nanospace, Polymer modification, SI-ATRP

INTRODUCTION

Recently, according to miniaturizing analytical space such as single cell analysis and proteomics, novel analytical device has been required for an extremely small volume of sample. Previously, we have focused on extended nanospace for solving this issue. We investigated that water showed unique properties such as high viscosity and high proton mobility, and developed novel analytical devices utilizing these properties [1]. For instance, we developed extended nanochromatography, which utilizes surface of extended-nano channel as stationary phase and achieves high separation capability for ultra-low volume of sample with large number of theoretical plates (~10^6 plate/m) [2].

In extended nanospace, surface of the channel affects largely on the chemical function due to large interfacial area. Surface modification method has been utilized to functionalize extended nanospace and control its effect. In extended nanospace, however, surface-modification method has been limited to low-molecular compounds, and polymer modification, which yields multi-functional surface, has not been achieved yet. The challenge is accurately regulation of density, orientation, and chain length on the surface for control of space size due to the size of conventionally-used polymer comparable to extended nanospace. The objective of our study is to develop polymer-modification method on extended nanospace utilizing SI-ATRP. Herein, we first report the availability confirmation of this SI-ATRP method onto microchannel. We next report polymer modification onto extended nanochannel and measurement of protein movement velocity in the extended nanochannel to investigate its function.

THEORY

To modify polymer onto surface of extended nanospace, we utilized SI-ATRP reaction [3]. Process of SI-ATRP reaction on the surface of extended nanochannel is shown in Fig. 1. First, initiator of polymerization, which is utilized as dormant P-X, is injected into extended nanochannel and immobilizes onto the surface utilizing silane-coupling reaction. Next, reaction solution of monomer and catalyst are injected into extended nanochannel. According to activation of initiator to the radical P· by catalyst, monomer units are added to the radical until it is deactivated back to dormant chain P-X. This cycle occurs repeatedly on the halogenated sites on the surface of the channel, thus allowing all graft chains to grow slowly and nearly simultaneously. Hence well-defined polymer surface which has regulated chain length (reaction velocity: several nm/h), high density, and high orientation is obtained. Therefore, by integrating this bottom-up polymer-modification method with top-down fabrication technique which creates extended nanochannel on quartz glass substrate, creation of regulated polymer-modified surface of extended nanochannel is possible.

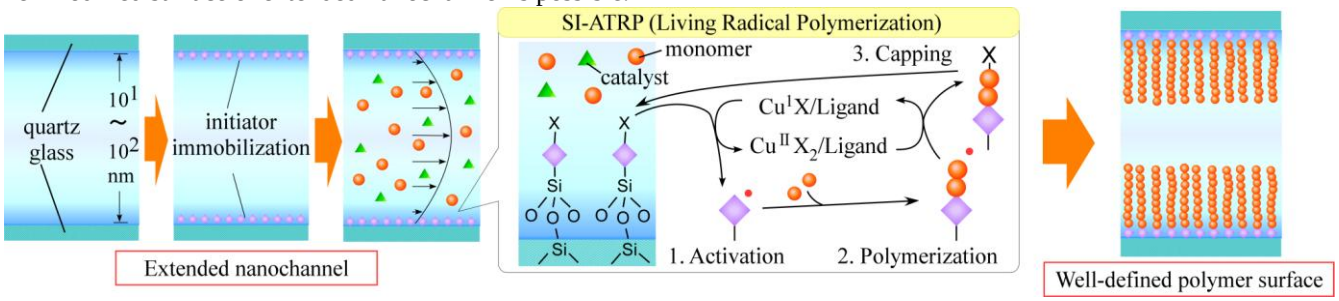

Fig. 1: Process of SI-ATRP reaction on the surface of extended nanochannel

EXPERIMENTAL

The extended nanochannels (1000 nm wide and 450 nm deep) were fabricated on quartz glass substrate by electron beam lithography and plasma etching. The microchannels (400 μm wide and 6 μm deep) and holes for introduction of reagent solution were also fabricated. And then, the substrate was thermally bonded with another substrate at 1080 ºC (Fig. 2).

Experimental setup for introduction of reagents into micro-nano chip is shown in Fig. 3. Initiator (1-trichlorosilyl-2-(*m,p*-chloromethylphenyl)ethane) was first immobilized on the surface of channel utilizing silane-coupling. Reaction solution of monomer (*N,N'*-dimethylacrylamide) and catalyst ($CuCl/CuCl_2$ and (tris[2-(dimethylamino)ethyl]amine) was introduced into the channel and SI-ATRP was performed.

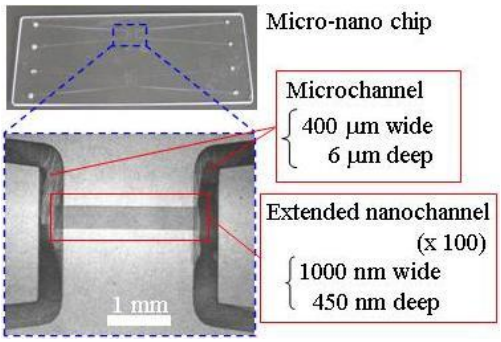

Fig. 2: Micro-nano chip

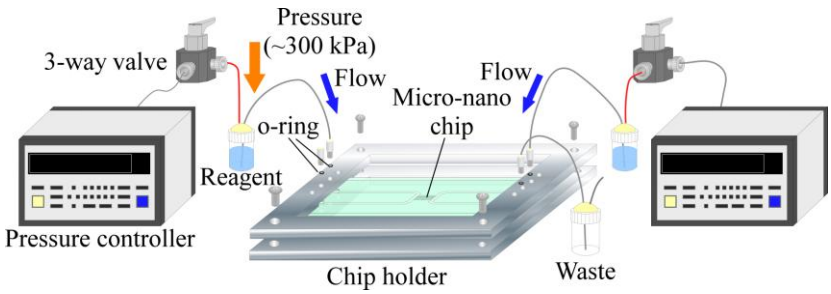

Fig. 3: Experimental setup

We adopted this process onto microchannel (400 μm wide and 2 μm deep) for availability confirmation of this method. Polymer modification on the surface of microchannel was evaluated by the resistance to protein adsorption [4]. Texas Red-labeled Bovine Serum Albumin (BSA) solution was introduced into unmodified and polymer-modified channels, which fabricated onto quartz glass chip without extended nanochannel. And then Phosphate Buffered Saline (PBS) was introduced for washing, and observed these microchannels by fluorescence microscope.

Next, the SI-ATRP method was adopted onto extended nanochannels and introducing Texas Red-labeled BSA into them. Measurement process of protein movement velocity in extended nanochannels is shown in Fig.4. The extended nanochannels and microchannels was first filled with PBS via left microchannel with high pressure (300 kPa), and then Texas Red-labeled BSA was introduced via right microchannel with low pressure (50 kPa). After releasing pressure of left channel and stopping flow, exit of right microchannel was capped and BSA was introduced into extended nanochannels with 300 kPa pressure. BSA movement was observed by fluorescent microscope and movement velocity was calculated.

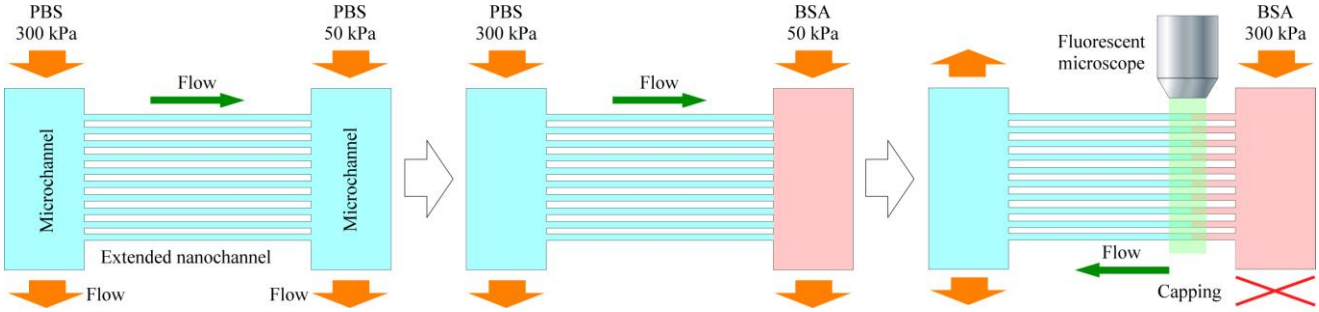

Fig.4: Measurement process of protein movement velocity in extended nanochannel

RESULTS AND DISCUSSION

We first confirmed availability of the SI-ATRP method on microchannel due to difficulty of evaluation on extended nanochannel. The result is shown in Fig.5. After washing unmodified and polymer-modified microchannel, reduction of fluorescent intensity was not observed on unmodified channel due to nonspecific adsorption of proteins. In contrast, fluorescent intensity was decreased on polymer-modified channel. These results indicate that resistance to protein adsorption is caused by polymer-modified surface. Therefore, we succeeded in polymer modification using SI-ATRP on quartz glass channel.

Next, we performed the SI-ATRP method on extended nanochannels and introduced BSA to investigate its function. The result is shown in Fig. 6. When Texas Red-labeled BSA was introduced into extended nanochannels, however, BSA did not completely filled extended nanochannels since movement velocity was very slow (~10^{-2} μm/s), even though PBS completely filled. In contrast, unmodified extended nanochannel was easily filled with BSA due to its high movement velocity

(~10^2 μm/s). These results indicate that large effect such as size exclusion was generated only after polymer modification and reduction of space size. Therefore, we suggested specific function in polymer-modified extended nanochannel.

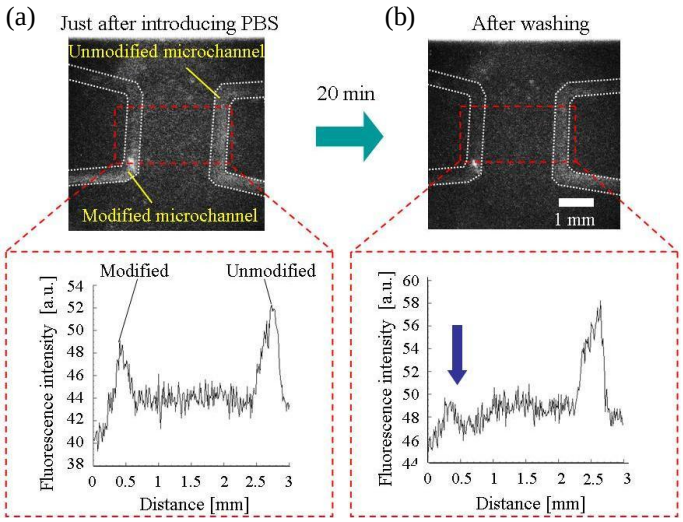

Figure 5: Fluorescence observation of absorbed Texas Red-labeled BSA in unmodified microchannel and microchannel modified with poly(N,N'-dimethyl acrylamide) (microchannel: 400 μm wide and 2 μm deep). Just after introducing PBS for washing (a), and after 20 min washing by PBS (b)

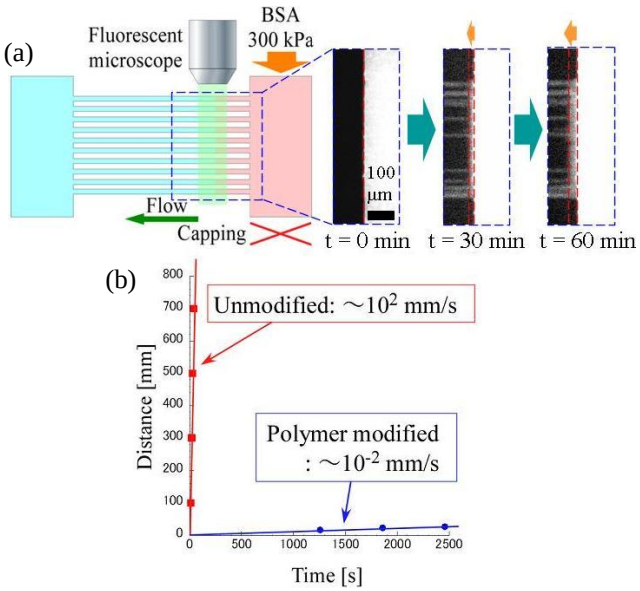

Figure 6: Measurement of BSA movement velocity in unmodified and polymer-modified extended nanochannel (1000 nm wide and 450 nm deep). Florescence observation of BSA movement (a), and comparison of BSA movement velocity (b)

CONCLUSION

We developed polymer modification method for creation of functional extended nanospace. We first confirmed availability of the SI-ATRP method on quartz glass microchannel. And then, we adopted the SI-ATRP method onto extended nanochannel and suggested expression of specific function. We believe that this method will yield novel biochemical analytical devices, such as protein-separation device for proteomics**.**

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