Study on Bead-based Microbiochip and Analytical System for Protein Detection

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Abstract - This paper presents bead-based microbiocihps to detect and separate target proteins. Micro beads coated with capture proteins were introduced into a microchamber, and target proteins flowing across the chamber were bound and concentrated. The chip was connected with an external fluid system. Bead surfaces were double-coated with photo-cleavable linkers and capture proteins. The proteins bound on the beads were photo-separated under UV irradiation, and excited to be measured in fluorescence. 38 ~ 50 ㎛ sized polystyrene beads were used. SOGs(silicon-on-glass) were used to fabricate the microchip having glasses bonded on both sides. 100 ㎛ thick silicon channel was formed through silicon deep RIE process. The upper glass cover had holed through to have inlets and outlets fabricated by powder-blastings. In this study, biotin and streptavidin were used as capture proteins and detection proteins, respectively. The protein mixtures of streptavidin, HSA(human serum albumin) and ovalbumin were applied for selective detection test.

1. Introduction

With development of micro fabrication technologies, various biological analysis procedures have been integrated into a single microfluidic device [1,2]. Microfluidic devices have been expected to reduce an amount of expensive reagents required and provide low-cost disposable chips with a high sensitivity and a high selectivity. Futhermore, the microfluidic devices can be equipped with analytical systems for mass spectrometry or fluorescence detection [3].

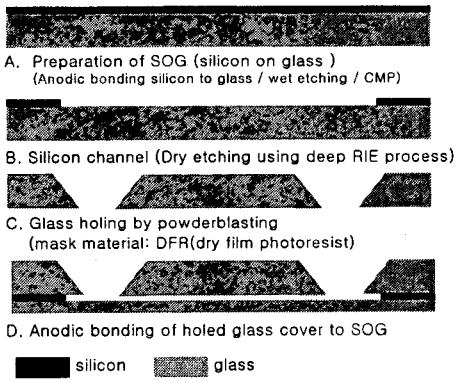
Biological samples are often present at too low concentration levels to analyze directly. In genetic analysis, fragmented and separated DNA molecules duplicate to be concentrated through the PCR (polymerase chain reaction) [4]. However, there are no generalized amplification methods in protein analysis. Since most biologically important proteins are macromolecules and given as mixtures, the specific surface modification protocols should be obtained to enhance a binding efficiency and a selectivity of target proteins and to minimize non specific bindings(NSB) [5]. In diagnostic analyses, the target molecules detection and separation from biological sample mixtures are followed generally by elution and concentration procedures. Since total amount of target molecules detected is very small, the detection region of substrate should be lowered to the order of sub-microlitter volume to obtain a small amount of purified proteins through elution. The selectivity and sensitivity of substrate, reaction area and volumetric resolution have compromising affects on analytic performances of biochips.

Recently, bead-based micro columns [6-8], complicated structures [9] and polymer porous media [10,11] are utilized for small volume detection and concentration. Especially, surface properties of microbeads are modified stably and easily in comparison with glasses, silicons and other materials. Beads can minimize reagent usages and provide enhanced surface area much more for chemical reaction to occur. They are also utilized as convenient mobile-phase reagents, which is convenient in on-chip and off-chip procedures from surface modification to protein-bound beads recollection.

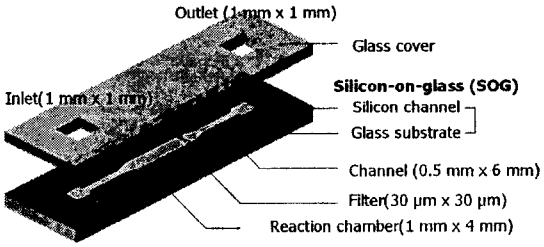
In this study, surface modified microbeads were used to chemically detect target proteins on beads. The microbeads were packed into a microchamber, which accommodated sub-micro litter liquid, to reduce reaction volumes. Analytical flow system was prepared with the microchip connected so that the bound proteins were separated from the beads and flowed out to fluorescence detection region. The amount of detected proteins were investigated in comparison with measured fluorescence, quantitatively. A bead-based analytic tool was presented for purification and separation of target proteins, bead affinity chromatography (BAC).

2. Fabrication of microchip

The layout of the microfluidic chip is shown in Fig. 1. The microfluidic chip was fabricated using silicon micromachining technologies. A silicon-on-glass (SOG) was used for channel fabrication. The microchip was finally composed of three layers, a glass cover, a silicon channel and a glass substrate with thicknesses of 500 ㎛, 100 ㎛, and 500 ㎛, respectively. The channel is transparent, so that observation during bead packing and sample injection and post-inspection on microscope are very easy. The silicon channel was formed in an SOG by standard photolithography and plasma reactive ion etching (RIE) processes. Two film masks were used for fabrication. The 16 silicon micro pillars (30 × 30 ㎛^{2}) was arranged in line with 30 ㎛ gap on an edge in a reaction chamber. The micro pillars were used as a filter for packing beads of the size from 38 ㎛ to 50 ㎛. The powderblasting was used to hole the cover glass for formation of an inlet and an outlet (1 × 1 ㎟),respectively. After cleaning and drying, the holed glass cover was bonded firmly to the SOG through anodic bonding for 2 hours. The glass substrate was electrically grounded to a metal plate heated by 400 °C, and 1 kV was applied to the glass cover.



(a) Fabrication processes



(b) Silicon microchannel in SOG, and glass cover

Fig. 1 Details of the fabrication processes and the fabricated microchip, and microscopic image of beads filter

3. Proteins detection principle

A fluorescence detection method was utilized as a protein analytic model. For a test, target proteins were coupled with fluorescence-tag (FITC-fluorescein isothiocyanate). The fluorescence excited by UV-irradiation on the detected target proteins was measured quantitatively. The streptavidin-biotin known was used as an analytic model for good reactivity and stability. The biotin was used as capture proteins for detection of the streptavidin. The polystyrene microbeads were pre-coated with the biotin, and introduced into the fabricated microchip as mobile substrates. Not only the target proteins but also physically trapped proteins may be excited by UV light simultaneously in case of on-beads fluorescence detection. The selectivity is also lowered, since other proteins can be non-specifically bound to the bead surface. To increase the selectivity, the target proteins on the microbeads should re-separated and flowed into another region for fluorescence measurements. Bead-based bioassay procedures are described in Fig. 2. The Fig. 2 (d) describes the fluorescence measurements during FITC tagged proteins passing by through silica-capillary tube. To enable on-beads re-separation of the target proteins, the beads were coated with photo-cleavable linker with the biotin (capture proteins). The chemical structures of the microbeads surface and the FITC are shown in Fig. 3.

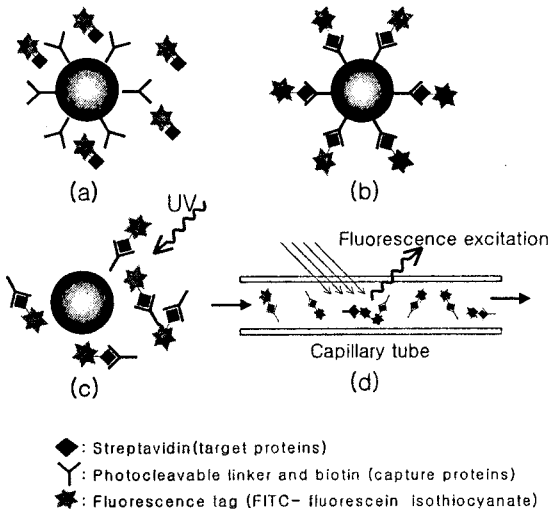
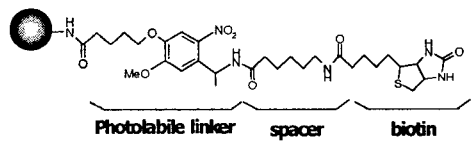


Fig. 2. General bead-based bioassay procedures from (a) to (d) in series: (a) protein mixtures injection into microchip, (b) states after selective binding of target proteins, and (if protein mixtures tested) washing-out of residual proteins, (c) photo-cleavage by UV irradiation, (d) fluorescence measurements on the eluted target proteins passing through capillary.



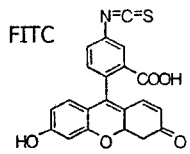
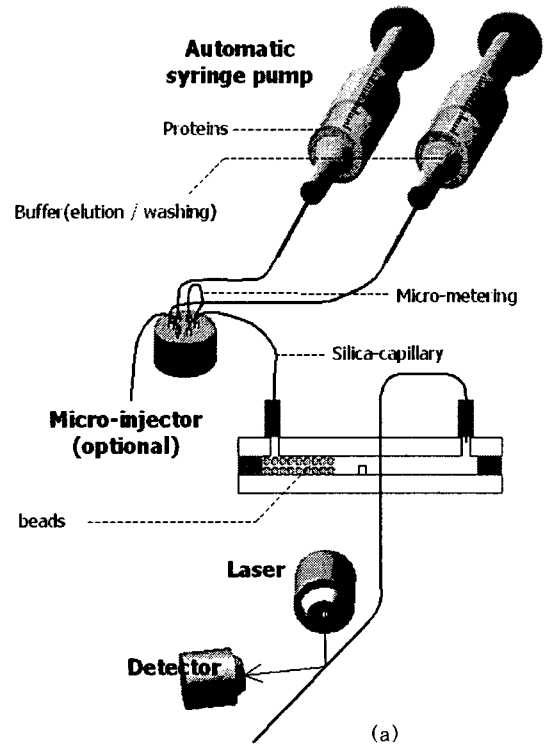
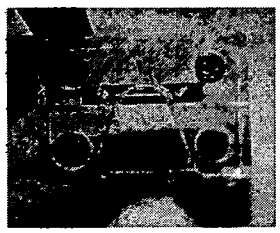


Fig. 3. Surface chemical structure of microbeads used for streptavidin detection and FITC

4. Analytical system

The fabricated microchip was equipped into the flow-based analytical system with a fiber optic UV set with a laser and a fluorescence detector as shown in Fig. 4. Automatic syringe pumps were used to control liquid at an adequate flow rate for cleaning, sample proteins injection, washing-out and elution. An acrylic jig was utilized to implement disposable treatments of microchips easily for introducing and recollecting microbeads across the chamber. An input pressure was measured to be near 70 kPa. The leakage by high pressure was prevented by using the capillary and acrylic jig combined system. It is also possible to UV-irradiate the beads for on-jig photo-cleavage of the target proteins bound biotin. The acrylic jigs were fabricated with two different tubes for bead packing and sample injection.





(b)

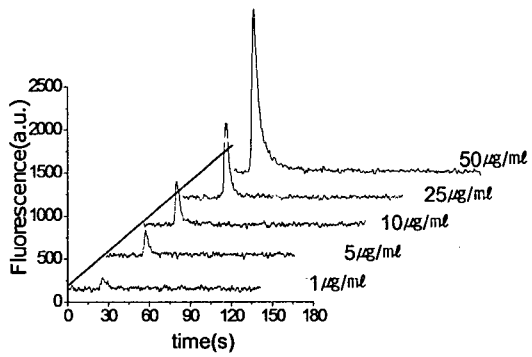
(c)

Fig. 4. Apparatus: (a) microchip equipped capillary-based flow system, (b) acrylic jig and (c) microscopic image of packed beads (bead size: 38 ~ 50 ㎛)

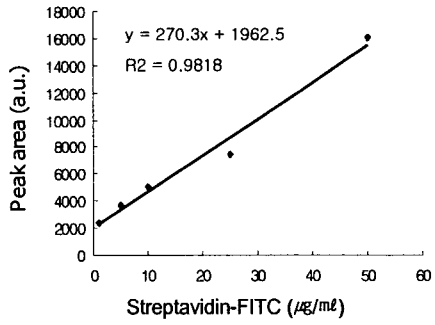
A difficulty related to bead based flow-system is a high pressure across the packed beads. However, by using the capillary tubes, micro-scale volume were controlled without any flow rate variations around the beads packed chamber. The capillary tubes were not deformed easily by an external pressure variation.

5. Experimentals

The differently concentrated streptavidin-FITC samples were prepared and tested to investigate the quantitative relation of proteins concentration with fluorescence excited by UV-irradiation, The experimental processes are as follows. 40 ㎕ of 0.33 % bead suspension, BSA(bovine serum albumin) blocking (20 min), 40 ㎕ of the bead suspension(0.33 % bead) was placed in the inlet tube of the BAC chip and packed successfully by suction at the outlet. To prevent NSB of the protein on the wall of the microstructure, the line and the bead, 0.5 % (w/w) of BSA solution was injected continuously by pumped flow (50 ㎕/min) for 30 min. And then, washing with phosphate buffer was performed by pumped flow (50 ㎕/min) for 10 min. Streptavidin-FITC or other protein sample (1 - 50 ㎍/㎖) was injected using the capillary syringe into the microchamber packed with the beads and incubated for 30 min. After the sample treatment, tween20 (0.5 % (w/w)/PB) was perfused into the bead-packed microchamber by pumped flow (50 ㎕/min) to wash out non-bound streptavidin- FITC. Washing was performed for 5 - 10 min until no signal were appeared by fluorescence detection at the capillary connected to the outlet of the microchip. The packed bead was irradiated by UV light (360 nm, 42 mW/㎠) using fiber optic UV set on the microchamber of the BAC chip. The phosphate buffer was perfused to elute the protein complex by pumped flow (50 ㎕/min). The eluted streptavidin-FITC complex could be detected by fluorescence detection. According to the standard curve, the eluted protein complex was analyzed quantitatively as shown in Fig. 5.



(a)



(b)

Fig. 5. Fluorescence measurement results about binding tests on different concentration of streptavidin sample: (a) electropherograms, (b) peak areas on each electropherogram

The streptavidin was also detected successfully from mixture of ovalbumin-FITC, HSA-FITC and streptavidin using the biotin-coated beads, as shown in Fig. 6. For the test, 0.16 μM streptavidin-FITC ,0.16 μM HSA-FITC and 0.96 μM ovalbumin-FITC is used with PB of pH 7.4, 50 mM. 40 ㎕ of 0.33 % bead suspension was packed. The time required for sample injection and reaction time was 30 min, and 10 min was required for UV-irradiation.

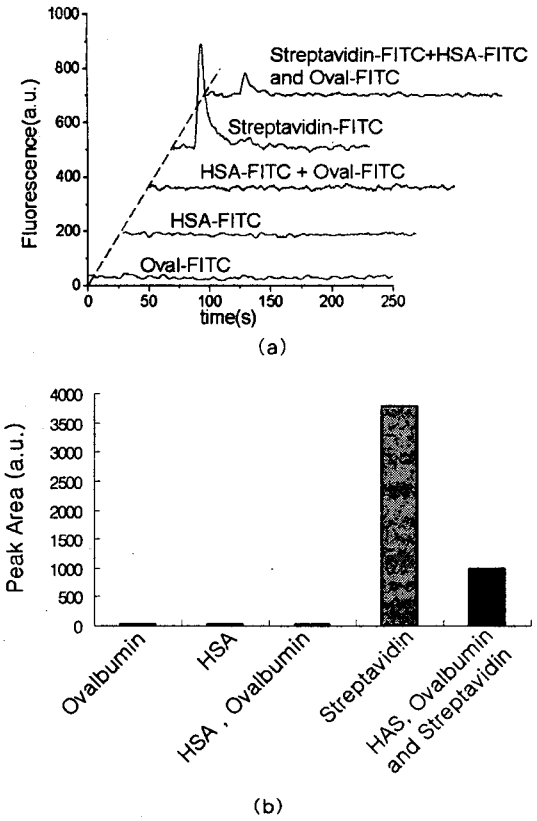


Fig. 6. Selective detection of streptavidin to HSA and ovalbumin: (a) electropherograms of detected streptavidin and (b) peak areas of each electropherogram

6. Conclusions

In this study, specific target proteins were detected using surface modified polystyrene microbeads of 38 ~ 50 ㎛. The microbeads coated with biotin(capture proteins) were used to detect the streptavidin (target proteins). A small amount of beads were successfully packed densely into the micro chamber of 1 ㎜ × 4 ㎜ × 100 ㎛. Although the pressure across the packed beads was measured so high near 70 kPa (0.7 atm), the leakage was prevented using capillary tubes based flow-system with acrylic jigs. The microchip was fabricated on SOG. On the SOG, another glass cover with an inlet and an outlet formed by powder-blasting was also anodic-bonded. The channel depth was measured near 100 ㎛. The pillar type silicon filters of 30 × 30 ㎛^{2} were formed in the micro chamber to the side of outlet to filter the beads. The streptavidin bound on beads is re-separated(elution) by photo-cleavage, and the eluted proteins was measured in fluorescence. For photo-cleavage, the photo-cleavable linker was chemically inserted between bead surface and biotin with spacer. The samples were combined with FITC for fluorescence detection.

In this study, the fluorescence was measured in proportion to the sample concentrations, and the streptavidin of 1 ㎍/㎖ as the smallest amount was detected. Finally, the streptavidin was selectively detected in protein mixtures of HSA, ovalbumin and streptavidin. Sample purification and separation of proteins could be made using this system, bead affinity chromatography (BAC).

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Acknowledgement

This research is supported by the Intelligent Microsystems Program (IMP) that is the project of the "21C Frontier R&D Program"