# DEVELOPMENT OF A MICROFLUIDIC BLOTTING DEVICE BY USING ALGINATE HYDROGEL

**Masahiro Ikawa[1], Yudai Fukushima[1], Kenji Sueyoshi[1], Fumihiko Kitagawa[2], Koji Otsuka[1]**

*[1]Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Japan, [2]Department of Frontier Materials Chemistry, Graduate School of Science and Technology, Hirosaki University, Japan*

## ABSTRACT

This paper proposes a concept of an easy-to-use microfluidic blotting device based on microscale electrophoresis. To realize the blotting assay with a simple experimental procedure, short analysis time, and high selectivity, alginate hydrogel was used as medium for a size-separation due to the molecular sieving effect and encapsulation of affinity ligands. In experimental, an alginate hydrogel was successfully generated in a microchannel by an electrokinetic introduction of $Ca^{2+}$. The formed hydrogels provided the size separation of DNA fragments and specific separation based on the affinity interaction, suggesting the applicability of the alginate hydrogel to the proposed device.

## KEYWORDS

Microfluidic blotting, microchip gel electrophoresis, affinity electrophoresis, alginate hydrogel

## INTRODUCTION

Recently, blotting assays for protein, DNA, and RNA play important roles in life science. In the conventional blotting assay, however, there are some disadvantages, *e.g.*, requirements of troublesome experimental procedures and long analysis time over a day, difficulty on the automation, and so on. To overcome these drawbacks, some groups have reported on developments of novel microfluidic blotting devices based on two-dimensional microchip gel electrophoresis (MCGE) [1], and MCGE in affinity ligand immobilized microchannels [2]. These devices easily provided the size separation of proteins and specific detection based on affinity trapping with short analysis time, while there still remains the requirement of bothersome procedures for the preparation of these devices. As a material to realize an easy-to-prepare blotting assay based on microfluidics, we focused on sodium alginate, which readily forms stable hydrogel by interacting with $Ca^{2+}$. In addition, the structure of the hydrogels can be controlled by changing the concentrations of alginate and $Ca^{2+}$, and the hydrogel has a high biocompatibility. In this study, we examined that an alginate hydrogel could be formed in a microchannel by an electrokinetic injection of $Ca^{2+}$. We also found that the formed hydrogel had the molecular sieving ability and could encapsulate an affinity ligand for the size separation of DNA fragments and affinity trapping of the molecules, respectively, to confirm a concept of a microfluidic blotting assay illustrated in Figure 1.

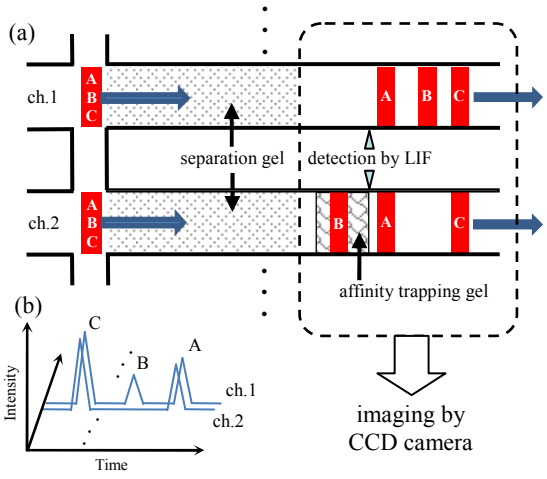


**Figure 1.** Concept of a microfluidic blotting assay in integrated channels filled with alginate hydrogels. (a) Illustration of integrated device containing different type of gels. (b) Expected electropherograms observed in each channels with different trapping gels by LIF detection.

## EXPERIMENT

In the microchip experiments, 50 mM HEPES buffer (pH 7.5) was selected as a background solution (BGS). YOYO-1 labeled DNA ladder (1–10 kbp) and λ-DNA (48.5 kbp) were used as test analytes. Figure 2 shows a preparation scheme of the alginate hydrogel in a microchannel. Briefly, the BGS containing 0.1% (w/v) sodium alginate was injected into a microchannel on a poly(methyl methacrylate) chip. Then, $Ca^{2+}$ was electrokinetically injected into the separation channel from a BGS waste (BW) reservoir, resulting in the on-line gelation of alginate. To confirm the on-line formation of the alginate hydrogel, YOYO-1 labeled λ-DNA and $Ca^{2+}$ were introduced into the separation channel filled with the BGS containing sodium alginate as shown in Figure 2c. To evaluate the separation efficiency of the formed hydrogel, the MCGE

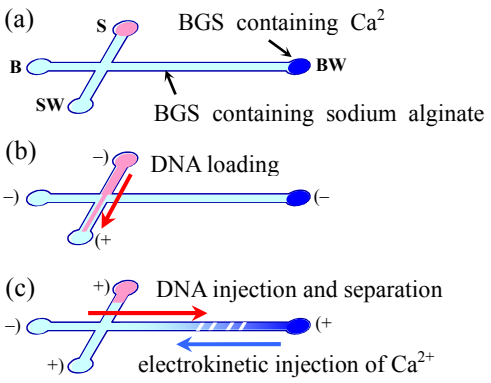


**Figure 2.** Schematics of the on-line gelation and DNA separation. (a) Initial condition, (b) DNA loading and (c) electrokinetic injection of $Ca^{2+}$ and DNA separation in the generated hydrogel. S, B, SW and BW are sample, sample waste, background solution (BGS) and BGS waste reservoirs, respectively.



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

analyses of 1 kbp DNA ladder with/without adding $Ca^{2+}$ were also carried out.

In capillary electrophoresis, P/ACE MDQ (Beckman Coulter) systems equipped with a LIF or UV absorbance detector were employed for the fundamental studies on the generation and characteristics of the hydrogel. As the hydrogelation in the microfluidic device, $Ca^{2+}$ was electrokinetically introduced into the capillary filled with a BGS containing sodium alginate, providing the capillary filled with the alginate hydrogel. To evaluate the applicability of the alginate hydrogel to an affinity separation, hydrogels with/without encapsulating avidin as an affinity ligand were formed by the electrokinetic introduction of $Ca^{2+}$ into the capillary partially filled with the BGS containing sodium alginate and avidin or no avidin. After the formation of the hydrogels, a sample solution was electrokinetically injected into the capillary, and then the separation voltage was applied to both ends of the capillary. As the test analytes, biotin and a mixture of 1, 5, and 10 kbp DNA fragments were used for evaluating the affinity of encapsulated avidin and size separation by the formed hydrogel, respectively

## RESULTS AND DISCUSSION

In microchip electrophoresis (MCE), λ-DNA rapidly migrated toward the anode by the electrophoresis with maintaining a spherical conformation as long as no $Ca^{2+}$ injection into the separation channel. By adding $Ca^{2+}$ into the BGS as shown in Figure 2a, on the other hand, the conformation of λ-DNA was changed from spherical to U-shape (Figure 3) with a remarkable deceleration of the electrophoretic migration of DNAs. These results indicate two important characteristics of alginate/alginate hydrogel: One is the less molecular sieving effect of the sodium alginate solution without $Ca^{2+}$. The other is that the hooking effect of the generated hydrogels, which might allow DNA fragments to be resolved according to their size by the molecular sieving effect.

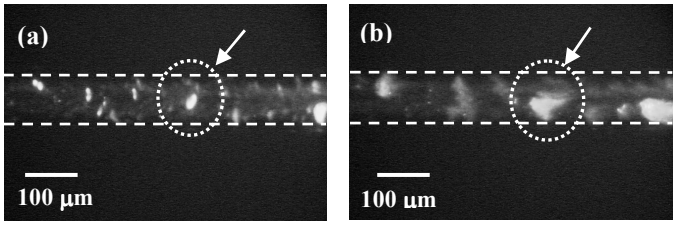


**Figure 3.** Variation of the conformation of λ-DNA during the electrophoretic migration. Fluorescence images were observed (a) 19 and (b) 25 s after injecting λ-DNA and $Ca^{2+}$ into the separation channel.

MCE analyses of 1 kbp DNA ladder were conducted in the separation channel with/without the alginate hydrogel to confirm the size-separation efficiency of the alginate hydrogel. The obtained electropherograms were depicted in Figure 4. As a typical result, the DNA ladder could not be separated in the sodium alginate solution (Figure 4a), whereas could be slightly resolved with the formation of the alginate hydrogel (Figure 4b). In addition, a couple of the trapped DNA bands were observed in the separation channel after the MCGE analysis of 1 kbp DNA ladder (Figure 4c), indicating the molecular sieving effect of the formed alginate hydrogel.

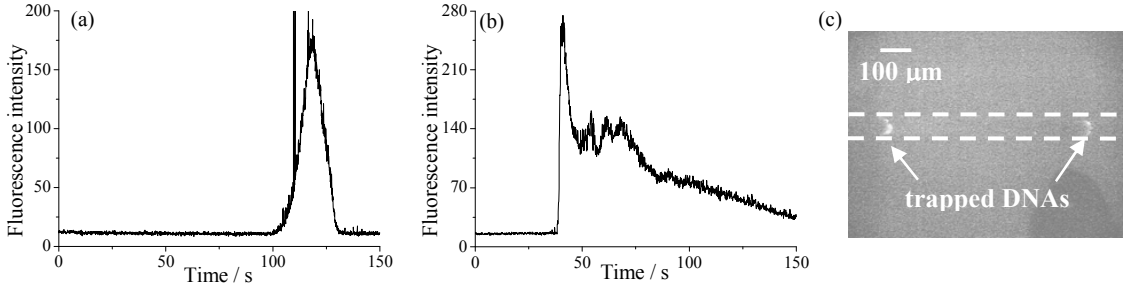


**Figure 4.** Effect of the on-line gelation on the separation of DNA fragments. (a, b) Electropherograms of 1 kbp DNA ladder without/with the on-line gelation of alginate, respectively. (c) Fluorescence imaging of the microchannel after the MCGE analysis with the on-line gelation. The BW reservoir was filled with (a) BGS and (b) BGS containing 10 mM $CaCl_2$, respectively. Separation length, 10 mm. Detection, LIF (ex/em = 488/520 nm).

To investigate the molecular sieving effect of the generated hydrogel in detail, electrophoretic analyses of DNA fragments were also carried out by using the alginate hydrogel-filled capillary. As a typical result, a mixture of 100, 500, and 1000 bp DNA fragments were not resolved at all in the capillary filled with a sodium alginate solution without the hydrogelation (Figure 5a). In the alginate hydrogel-filled capillary, on the other hand, 500 and 1000 bp DNAs were well resolved, whereas 100 and 500 bp DNA fragments were not. These results also indicated the capability of the alginate hydrogel as a molecular sieving medium, while a significant band broadening was also observed as shown in Figure 5b. This band broadening suggests the inhomogeneous structure of the alginate hydrogel, so that the protocol of the hydrogelation has to be optimized to achieve further effective separations of the complicated DNA mixtures.

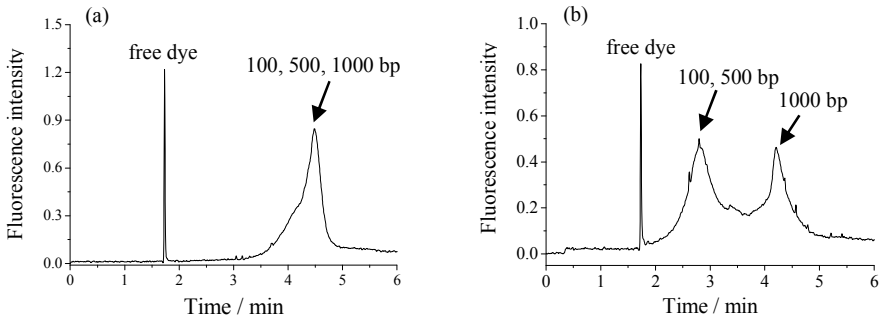


**Figure 5.** Electropherograms of DNA mixture obtained by using the capillaries filled with (a) sodium alginate solution and (b) alginate hydrogel, respectively. BGS, 0.1% sodium alginate prepared with (a) 25 mM HEPES buffer (pH 7.5), (b) 5 mM $CaCl_2$, 25 mM HEPES buffer (pH 7.5); sample, 5.0 ng/μL 100, 500, and 1000 bp DNA fragments labeled with YOYO-1 prepared with 25 mM HEPES buffer (pH 7.5); capillary, fused silica capillary (50 μm i.d., total/effective length, 40/30 cm); sample injection, pressure (0.3 psi, 5 s); detection, LIF (ex/em = 488/520 nm).

To realize the proposed blotting device, it was also required to evaluate the encapsulation of affinity ligands by the alginate hydrogel with maintaining their affinities. Avidin, which has a strong affinity of interacting with biotin, was selected as a test ligand. A sodium alginate solution containing fluorescein isothiocyanate (FITC)-labeled avidin was partially introduced into the capillary filled with the BGS containing $Ca^{2+}$. After applying a voltage to the capillary, the fluorescence from FITC-avidin was only observed at the partially filled hydrogel zone formed by the addition of $Ca^{2+}$, indicating the encapsulation of avidin in the hydrogel. Affinity capillary electrophoresis (ACE) of biotin was also conducted to clarify that the alginate could maintain the affinity of the encapsulated avidin. As a typical result, biotin was detected in the prepared capillary partially filled with hydrogel without avidin, whereas not observed in that encapsulating avidin (Figure 5). Hence, it was found that the alginate hydrogel could encapsulate the affinity ligands with maintaining their affinity without any difficult/troublesome procedures [1–3].

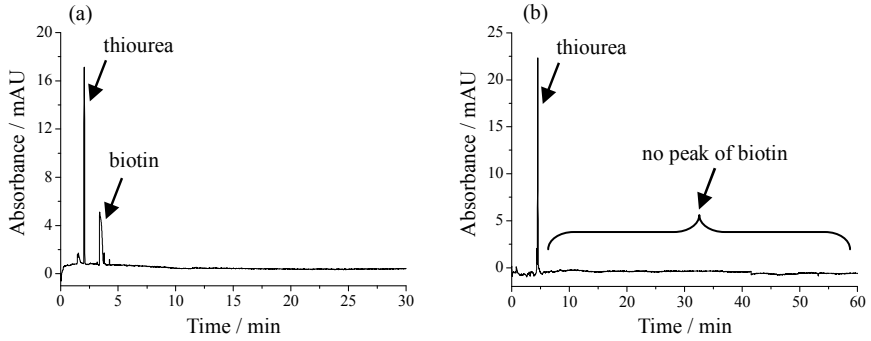


**Figure 6.** Affinity electrophoresis of biotin by using the capillaries partially filled with the alginate hydrogel encapsulating (a) no avidin and (b) 0.3 mg/mL avidin. BGS, 1 mM $CaCl_2$ / 15 mM HEPES buffer (pH 7.0); sample, 50 ppm thiourea, 200 ppm biotin / BGS; capillary, fused silica capillary (50 μm i.d., total/effective length, 40/30 cm); sample injection, electrokinetic (5 kV, 20 s); detection, UV absorption (200 nm).

Consequently, these results indicate a feasibility of alginate hydrogel to the proposed microfluidic blotting device integrated with MCGE and ACE. The multiplication of the channels filled with the hydrogels is in progress for the realization of the proposed microfluidic blotting device.

## REFERENCES

[1] M. He, A. E. Herr, *Polyacrylamide Gel Photopatterning Enables Automated Protein Immunoblotting in a Two-Dimensional Microdevice,* J. Am. Chem. Soc., 132, pp. 2512–2513 (2010).
[2] W. Pan, W. Chen, X. Jiang, *Microfluidic Western Blot*, Anal. Chem., 82, pp. 3974–3976 (2010).
[3] K. Sakai-Kato, K. Ishikura, *Integration of Biomolecules into Analytical Systems by Means of Silica Sol-Gel Technology*, Anal. Sci., 25, pp. 969–978 (2009).

## CONTACT

Masahiro Ikawa, +81-75-383-2449 or ikawa.masahiro.67c@st.kyoto-u.ac.jp