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이학석사학위논문

On-line Immunassay of a Cardiac Biomarker in Serum Using a Polyester-Toner Microfluidic Device

폴리에스터-토너 마이크로 유체칩을 이용한
칩 상에서의 혈장에 있는 심장질환 생체지표 면역분석법

2013년 2월

서울대학교 대학원
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2013년 2월

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2013년 2월

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Abstract

On-line Immunassay of a Cardiac Biomarker in Serum Using a Polyester-Toner Microfluidic Device

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An on-line immunoassay to detect C-reactive protein (CRP) was performed using a polyester-toner (PT) microfluidic device. CRP is a highly conserved plasma protein responding to inflammation and is used for clinical purposes to diagnose an inflammatory state. For rapid analysis and specific interactions in immunoassays, extensive studies using microfluidic chips have been carried out. Recently, a simple technique to fabricate a disposable PT microchip based on a

direct printing process was developed and several applications were introduced. One major drawback of the PT microchip, however, is the poor separation performance due to the quality of the microfluidic structures. This problem for a PT microchip can be overcome using a cleavable tag immunoassay, which requires minimal separation performance. After analytes are conjugated onto antibodies which are immobilized on the surface of microbeads placed on the PT microchip, a second group of fluorescently tagged antibodies is added and complexed with the analytes. The tag is then cleaved and the solution containing the cleaved tag is analyzed by electrophoresis. The time needed for the complete analysis to be carried out on a PT microchip was less than 35 min. The dynamic range of the CRP in 10-fold diluted serum was 0.3 to 20 mg/L and the limit of detection was about 0.3 mg/L, which demonstrated the possibility of a quantitative analysis of CRP in serum in clinical trials.

Keywords:

Polyester-toner microchip, microfluidic immunoassay, C-reactive protein, cleavable tag immunoassay

Student number: 2010-20266

CONTENTS

ABSTRACT i

CONTENTS iii

LIST OF FIGURES v

LIST OF TABLES vi

1. INTRODUCTION 1

2. EXPERIMENTAL 3

- **2.1 Materials and reagents** 3
- **2.2 Synthesis of the labeled detection antibody** 4
- **2.3 Immobilization of the capture antibody onto silica microbeads** 5
- **2.4 PT microchip fabrication** 6
- **2.5 Sandwich immunoassay of CRP** 9

3. RESULT AND DISCUSSION 14

3.1 Characteristics of a PT microchip 14

3.2 Analytical performance of the on-chip sandwich immunoassay 14

3.3 On-chip sandwich immunoassays in human serum samples 23

4. CONCLUSIONS 26

REFERENCES 27

ABSTRACT IN KOREAN 30

LIST OF FIGURES

Figure 1. Schematic of the PT microchip fabrication process and layout. 8

Figure 2. Immunoassay procedure. 13

Figure 3. Electropherograms of off-chip CTI with a commercial CE instrument and on- and off-chip CTI with a PT microchip. 18

Figure 4. Immunoreaction time versus the peak height of CRP (10 mg/L). 20

Figure 5. Electropherograms of CRP and a control, IgG. 21

Figure 6. Calibration curve of the CRP concentration in the blocking buffer versus the peak height. 22

Figure 7. Calibration curve of spiked CRP in serum versus the peak height. 24

Figure 8. Electropherograms: CRP (10 mg/L) in the blocking buffer and spiked CRP (10 mg/L) in serum versus the peak height. 25

LIST OF TABLES

Table 1. High-voltage program used in the on-chip immunoassay 12

Table 2. Analytical performance of CTI with a commercial CE and with or w/o PT microchip 19

1. Introduction

Over half of all cardiovascular-related diseases do not show early symptoms. Therefore, biomarkers are used as pre-diagnostic indicators.[1] C-reactive protein (CRP) is a highly conserved plasma protein responding to inflammation, and an increase in its plasma concentration is clinically considered to indicate an acute inflammatory state.[2] The Centers for Disease Control and the American Heart Association acknowledged the correlation between a CRP increase in serum and future cardiovascular events and thus endorsed CRP as an inflammatory marker for clinical diagnoses of cardiovascular diseases such as myocardial infarction, ischemic stroke, and sudden cardiac death.[3] About a quarter of the US population has plasma CRP levels higher than 3 mg/L, and a CRP level higher than 10 mg/L may reflect an acute phase of a cardiovascular disease that would call for a closer investigation with repeated tests.[2, 4]

In clinical laboratories, the CRP level has usually been measured by an immunoassay due to the outstanding specificity of antigen/antibody interactions.[5] Conventional immunoassays are carried out in microwell plates requiring a long reaction time with labor-intensive manual procedures or an expensive robotic system.[6] When a microfluidic device with integrated sample treatment steps is used, the immunoreaction time, labor, and consumption of the sample and reagents can be greatly reduced.[7-15] Furthermore, microfluidic devices are

promising tools for high throughput assays and good portability.[16]

Microfluidic devices for immunoassays are fabricated using various materials, including silicon, silicon nitride, glass, quartz, metal, polystyrene, cyclic polyolefin, PDMS, and PMMA.[17] The fabrication processes, however, are usually complicated and require special facilities. Recently, a very simple process of fabricating a polyester-toner (PT) microchip by direct printing on a transparent polyester film using an office-type laser printer was introduced, and various applications, such as microchip electrophoresis[18-24], a micromixer[25] and a microreactor[26] have been demonstrated. In this work, we fabricated a PT microchip capable of performing a cleavable tag immunoassay (CTI)[27] and used it to analyze CRP in a serum sample. In the CTI, only the fluorescent tag cleaved from the detection antibody was injected into the electrophoresis channel. Therefore, we were able to overcome the low separation quality associated with a typical PT microchip. The overall analysis was completed within 35 min. The dynamic range, limit of detection (LOD), and limit of quantification (LOQ) of CRP in a 10-fold diluted sample of serum were 0.3 to 20 mg/L, ~0.3 mg/L, and ~1 mg/L, respectively, showing the capability of clinical analyses of CRP in an actual sample.

2. Experimental

2.1 Materials and reagents

Polyethylene terephthalate (PET) transparency films (CG3300) were from 3M (Austin, TX, USA). Sulfo-NHS-SS-biotin, sulfo-NHS-biotin, and tris(2-carboxyethyl)phosphine (TCEP, 0.5 mM) were purchased from Thermo Pierce (Rockford, IL, USA). Non-porous silica microbeads (100 mg/mL) with a diameter of 2.56 μm were from Bangs Laboratories (Fishers, IN, USA). 3-Aminopropyltriethoxysilane (APTES, 99%), boric acid, fluorescein-5-isothiocyanate (FITC), 25 wt% aqueous solution of glutaraldehyde, hexamethylenediamine, sodium chloride, sodium tetraborate, triethylamine (TEA), ethanol, avidin from egg white, CRP from human plasma, 29 wt% aqueous ammonia solution, phosphate -buffered saline (PBS) dry powder, Tween 20, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Monoclonal antibodies to CRP produced in mouse (clones 5 and 7) were from Exbio (Praha, Czech Republic). CRP-free human serum was from Sunny Lab (Sittingbourne, UK). Methanol was from Merck (Darmstadt, Germany) and tris(hydroxymethyl) aminomethane (Tris) was from ICN Biomedicals (Aurora, OH, USA).

The borate buffer used for the antibody immobilization reaction and electrophoresis was prepared by titrating 20 mM sodium tetraborate with 1 M

NaOH to pH 9.2 at 25°C. 1× PBS was prepared by dissolving PBS dry powder in water and titrating it to pH 7.4 with 1 M HCl. A stock solution of CRP as a model analyte was prepared in a PBS-Tween 20 mixture (PBS-T, 1× PBS with 0.05 vol% Tween 20). PBS-T and borate buffer were used for washing during the on-chip immunoreaction. To prevent the non-specific binding of serum proteins, a blocking buffer (PBS-T with 0.01 wt% BSA) was used before the CRP-antibody binding reactions and for the dilution of the serum.

2.2 Synthesis of the labeled detection antibody

10 mg of FITC and 20 mg of hexamethylenediamine were reacted overnight at room temperature in 1 mL of methanol containing 10 μL TEA to prepare fluorescein thiocarbamyl hexamethylenediamine (FTHD).[28] After the reaction, the precipitated product was washed with 1 mL of methanol/acetonitrile (1:10 vol/vol) and dried under ambient conditions. The precipitate was then reacted with 16 mg sulfo-NHS-SS-biotin (~1:1 molar ratio with FTHD) in 1 mL 1× PBS for 4 h at 4°C to synthesize the tag, fluorescein hexamethylenediamine biotin (FHB). 100 μL of 1 mg/mL CRP detection antibody (clone 5) in 1× PBS was biotinylated with 50 μL of 100 μg/mL sulfo-NHS-biotin in 1× PBS for 2 h at 4°C. The biotinylated detection antibody in 1× PBS was then reacted with 150 μL of 5 mg/mL avidin in 1× PBS for 2 h at room temperature. The molar ratio

between the biotin and the avidin was 1:1. Subsequently, 450 μL of 25-fold diluted FHB solution in 1× PBS was added to the avidin-biotinylated detection antibody and bound to the remaining sites of the avidin at 4°C overnight. The reaction products were used as detection antibodies.

2.3 Immobilization of the capture antibody onto silica microbeads

50 μL of a 100 mg/mL silica microbead solution was suspended in 1 mL of ethanol. 60 μL of 29 wt% ammonia solution and 50 μL APTES were added to the suspension and the mixture was agitated overnight at room temperature. The microbeads were separated by centrifugation at 1100*g* for 60 s, washed three times with 1 mL of borate buffer, and suspended in 1 mL of 0.25 mM Tris solution containing 10 μL of a 25 wt% glutaraldehyde solution. The suspension was stirred for 2 h at room temperature and the reacted microbeads were collected by centrifugation and washing with a borate buffer as above. The glutaraldehyde-modified microbeads were suspended in 1 mL of 1× PBS. After adding 10 μL of 1 mg/mL CRP capture antibody (clone 7), the mixture was stirred for 1 h at room temperature. The capture antibody-immobilized microbeads were washed three times with 1× PBS and then centrifuged at 1100*g* for 60 s, dispersed in 1 mL of 1× PBS, and stored at 4°C until their use.[28]

2.4 PT microchip fabrication

The PT microchip fabrication procedure was previously reported in detail[18] and will be summarized here briefly (Fig. 1). A microchip layout was prepared using Adobe Illustrator CS5 (Adobe Systems, San Jose, CA, USA). The channel width was 200 μm, the electrophoresis channel was 16.0 mm long, and other connecting channels were 8.0 mm long. A double-T injector with a 450 μm offset for the pinched injection was located between the sample reservoir (SR) channel and the sample waste reservoir (SW) channel. Three weirs each with a width of 120 μm were prepared at the SR outlet in order to prevent leakage of microbeads from the SR to the connecting channels during the assay. An opening of 80 μm was formed on the channel wall so that fluid could flow through whereas the microbeads could not. The layout was printed on a polyester transparency film using an HP LaserJet 2100 (Hewlett-Packard, Boise, ID, USA) with a C4096A toner cartridge at a maximum resolution of 1200 dots per inch. The printed transparency film and a blank polyester film were then subjected to a corona discharge treatment[29] using a Tesla coil (BD-10A; Electro-Technique Products, Chicago, IL, USA) under the condition of 35–40% humidity so that the channel surfaces were made hydrophilic for easy injection of the run buffer and sample. The printed transparency film and the blank film were then joined using a heating laminator (SKY-325R6, Peach Laminating, Seoul, Korea) at 130°C and at

a feeding rate of 1000 mm/min to form channels in the blank regions. Following the lamination step, 200 μL pipette tips were attached using hot melt stick glue (Keumsung, Daejeon, Korea) for use as liquid reservoirs. The chip fabrication process was completed within 15 min, from printing to the reservoir preparation step.

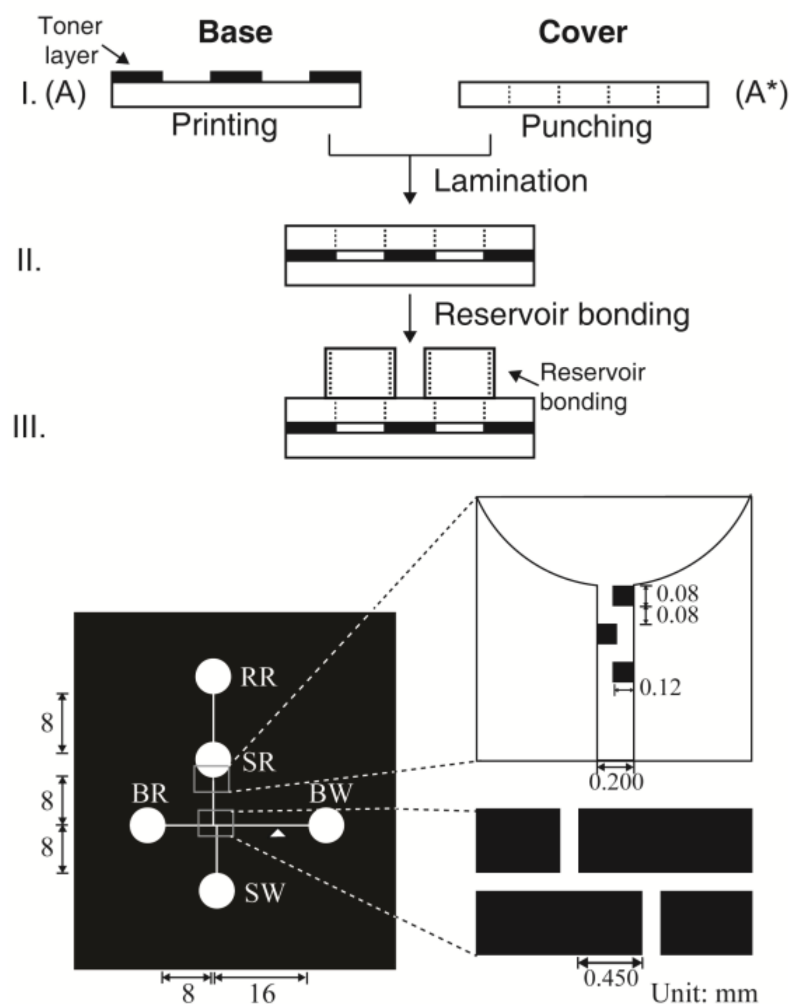

Figure 1. Schematic of the PT microchip fabrication process and layout. **I.** (A) Base film of a PT microchip created by a laser printer, (A*) punching the reservoir area of the cover film of a PT microchip; **II.** Lamination; **III.** Reservoir bonding. Abbreviations represent the following terms: sample reservoir (SR), sample waste reservoir (SW), buffer reservoir (BR), buffer waste reservoir (BW), and rinse reservoir (RR). The triangle located 10 mm from the double-T injector toward the BW represents detection point.

2.5 Sandwich immunoassay of CRP

A PT microchip was placed on a microscope (IX71, Olympus, Tokyo, Japan). After hydrodynamically filling the channels of the PT microchip with borate run buffer, the flow of the liquids was electrokinetically controlled using a high-voltage power supply (DBHV-100, Digital Bio Technology, Seoul, Korea) connected to an IBM-compatible PC through a COMI-CP301 D/A board (COMIZOA, Daejeon, Korea). The sandwich immunoassay of the CRP consisted of two parts: CTI and electrophoresis. The voltage programming of each step is given in Table 1.

The procedures for the on-chip CTI were adapted from earlier work[28], as illustrated in Fig. 2. 30 μL of the suspension of capture antibody-immobilized microbeads in 1× PBS was injected into the SR. After rinsing the microbeads in the SR three times each with 100 μL of PBS-T using a micropipette, 45 μL of CRP sample solution was added to the SR and the reaction was carried out for 10 min at room temperature. The electroosmotic flow (EOF) was induced from the SR toward the rinse reservoir (RR) by applying lower voltage to the RR compared to that applied to other reservoirs. After the reaction, the solution in the SR was removed and rinsed five times each with 100 μL of PBS-T, as described above. During the rinsing procedure, a constant potential from the SR to the RR was applied to remove unbound reactants. 90 μL of the detection antibody solution (~1

mM) with a cleavable tag, which was double that of the analytes, was added to the SR. This step was followed by a reaction for 10 min at room temperature. Unreacted detection antibodies also migrated toward the RR due to the EOF during the reaction. After the reaction, the rinsing procedure was repeated 5 times each with 100 μL of a run buffer. The SR was then filled with 100 μL of run buffer containing 5 vol% TCEP and the cleavage reaction was carried out for 10 min. The fluorescent tag from the detection antibody was cleaved with TCEP by reducing the disulfide bond in the tag moiety. As a result, the cleaved tag was dispersed in the sample solution while the antigen and the detection antibody were immobilized on the microbeads.

The cleaved tag was loaded into a double-T injector by flowing the solution in the SR toward the SW, after which electrophoresis with laser induced fluorescence (LIF) detection was carried out. Through a 10× objective of a microscope, the excitation beam from an argon-ion laser (488 nm, 10 mW, Melles Griot, Carlsbad, CA, USA) was loosely focused onto the detection point located 10 mm from the double-T injector toward the buffer waste reservoir (BW) covering the full width of the channel. The fluorescence emission was filtered through a 520-nm band pass filter (CVI, Albuquerque, NM, USA), focused by the same objective and then detected using a photomultiplier tube module (PMT; HC 120-01, Hamamatsu, Bridgewater, NJ, USA). The PMT signal was collected and processed using a PCI-MIO-16XE-50 board (National Instruments, Austin, TX,

USA) on an IBM-compatible PC controlled with in-house software written using LabVIEW 7.0.1 (National Instruments).

As a reference, off-chip CTI was also carried out in the same manner used with the on-chip CTI process except for immunoreactions of 2 h each and the collection of the cleaved tag with a 3 kD Nanosep centrifugal concentrator (Pall Corporation, Port Washington, NY, USA). The cleavage tag was then analyzed either with a PT microchip as described above or with a commercial CE instrument (P/ACE MDQ, Beckman Coulter, Fullerton, CA, USA). For conventional CE, a bare fused capillary (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm ID and with a total/effective length of 40/30 cm was rinsed with 0.1 M NaOH, water, and borate run buffer for 3 min, each at 80 psi. A reverse potential of –20 kV was applied across the capillary, which was maintained at 25°C, and the tag was monitored with LIF.

Table 1. High-voltage program used in on-chip immunoassay

	Voltage (kV)					Duration time (s)
	Rinse reservoir	Sample reservoir	Buffer reservoir	Sample waste reservoir	Buffer waste reservoir	
Reaction	0.0	0.01	0.01	0.01	0.01	2000
Injection	0.0	0.05	0.1	0.2	1.0	60
Electrophoresis	0.5	0.6	0.1	1.5	0.6	40

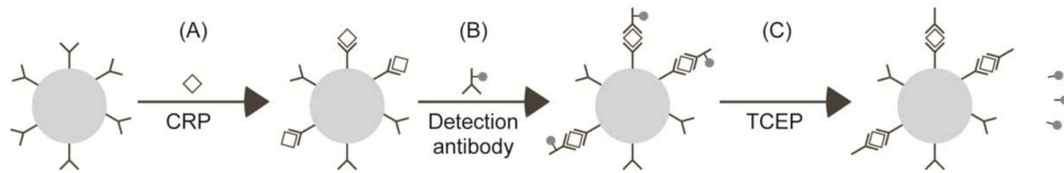

Figure 2. Immunoassay procedure. (A) Add CRP to the antibody-immobilized bead solution for the reaction. (B) Add detection antibodies with fluorescent tags. (C) Add TCEP to cleave the tags.

3. Results and discussion

3.1 Characteristics of a PT microchip

For the microfluidic immunoassay on a PT microchip, several features were implemented. First, a rinse reservoir (RR) was added to the SR. During the reaction steps, a lower potential was applied to the RR than the other reservoirs to prevent the leakage of reactants and products into the separation channel. Second, a single-toner layer chip was used instead of the usual double-toner layer chips fabricated by laminating two polyester films printed with a layout of microchannels and its mirror image. This was done to reduce the risk of bubble formation caused by Joule heating accumulated from the long application of electric potentials during several reaction steps. Due to the smaller cross-sectional areas of microchannels, a single-toner layer chip produces less Joule heating and thus fewer bubbles. Moreover, the chip fabrication process was simplified since the exact alignment of the channel structures printed on the two polyester films was unnecessary. Finally, to prevent the leakage of microbeads from the SR, weirs were added at the outlet of the SR, which was done simply by drawing additional lines in the chip layout area.

3.2 Analytical performance of the on-chip sandwich immunoassay

The EOF profiles in the microchannels of the PT microchip are not flat,

as the top and bottom surfaces of the microchannel consist of polyester films and the sidewalls of the microchannel consist of toner materials. In addition, the channel surfaces are rough and a number of random toner spots exist in the channels due to the limitation of the printing process by a laser printer. Therefore, the separation quality obtainable with a PT microchip is much worse than that from a quartz, glass, or polymer microchip. As a result, it is quite difficult to carry out an immunoassay of a biological sample in a complex matrix on a PT microchip. One remedy to this problem is CTI.[27] When using this method, a fluorescent tag detached from the detection antibody was injected into the electrophoresis channel, while all other components remained immobilized on the microbeads or were removed by rinsing. The difficulty of protein separation could therefore be avoided and CRP in serum could be assayed using a PT microchip.

In order to confirm whether the immunoreactions proceeded well on a PT microchip, the electrophoresis results of cleaved tags obtained through off-chip immunoreaction were compared with the on-chip immunoassay result. Fig. 3A shows an electropherogram of a tag from off-chip CTI obtained with a commercial CE instrument. Figs. 3B and 3C show electropherograms with PT microchips of tags from off-chip CTI and on-chip CTI, respectively. The signal to noise ratio (S/N, n = 3) and the number of plates (N) (n = 3) for the peak of tag obtained with the commercial instrument, off-chip CTI, and on-chip CTI were (14000, 13000), (270, 680), and (100, 600), respectively. The relative standard

deviations of the migration time and peak height were (3%, 4%), (17%, 16%), and (23%, 26%), respectively for the commercial instrument, off-chip CTI, and on-chip CTI. As expected, the quality of the CE results with the PT microchips was greatly inferior to that from the commercial CE instrument. However, the on-chip CTI results obtained within 35 min were quite comparable to those from off-chip CTI obtained within 4.5 h, demonstrating the feasibility of the sandwich immunoassay on a PT microchip.

As shown in Fig. 4, the peak height of the cleaved tag was increased with the reaction time. In the on-chip immunoassay, however, when the electric field was applied to the chip for over an hour, the flow was interrupted since bubbles had been formed in the channel due to the heat buildup. Therefore, the entire reaction time was limited to 50 min.

The specificity of our immunoassay was checked using IgG (pI 7.3[30]) as a control. Since either IgG or the detection antibody could not be immobilized on the microbeads and migrated to the RR by EOF, cleaved tags were not detected in the electrophoresis channel, as expected. The interference of IgG was also tested using a mixture sample of CRP and IgG. Fig. 5 shows that our sandwich immunoassay responded only to CRP.

In addition, in order to examine the quantitativity of CRP analysis, the on-chip immunoassay process was carried out with CRP samples of different concentrations. The dynamic range of CRP was 0.3 to 100 mg/L (Fig. 6). The

calibration curve for CRP using a standard four-parameter logistic curve (4PL) takes the following equation:[31]

$$y = \text{Min} + (\text{Max} - \text{Min}) \Big/ \left[1 + \left(\frac{x}{\text{EC}_{50}} \right)^{B} \right] \tag{1}$$

The curve was plotted with a logarithmic-scale x-axis with the Min and Max being the minimum and maximum asymptotes of the curve, with the half-maximal effective concentration (EC_{50}) serving as the inflection point on the calibration curve and B representing the slope factor. The parameter values obtained from fitting to the data were as follows: Min = 0.030 ± 0.001, Max = 1.522 ± 0.003, EC_{50} = 10.602 ± 0.046, B = –1.378 ± 0.008 and r^2 = 0.999 (n = 8). The LOD (S/N = 3) and LOQ (S/N = 10) for CRP in blocking buffer were ~0.3 mg/L and ~1 mg/L, respectively. The migration time was approximately 10 s ± 3.

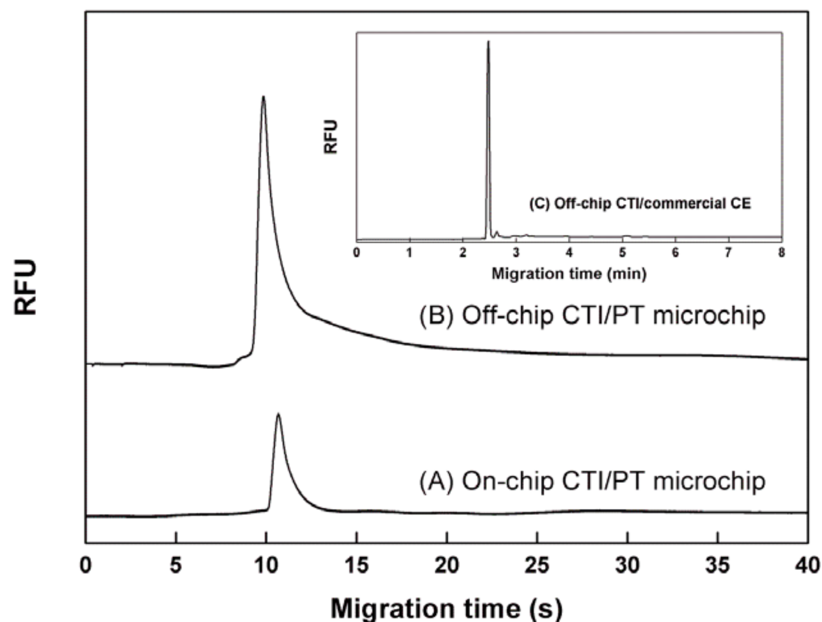

Figure 3. Electropherograms of off-chip CTI with a commercial CE instrument and on- and off-chip CTI with a PT microchip. (A) Off-chip CTI of CRP (10 mg/L) using a commercial CE instrument. (B), (C) Off-chip CTI and on-chip CTI of CRP (10 mg/L) using a PT microchip. Electrophoresis conditions of a commercial CE instrument: 50 μm ID, 40/30 cm fused silica capillary; run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm; –20 kV at 25°C. Electrophoresis conditions of the PT microchip: run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm. Voltages were programmed as shown in Table 1.

Table 2. Analytical performance of CTI with a commercial CE and with or w/o PT microchip

	Number of plate (N)	Siganal to noise ratio (S/N)	RSD (%, n = 3)	
			Duration time (s)	Peak height
Off-chip CTI/commercial CE	14000	13000	3	4
Off-chip CTI/PT microchip	270	680	17	16
On-chip CTI/PT microchip	100	600	23	26

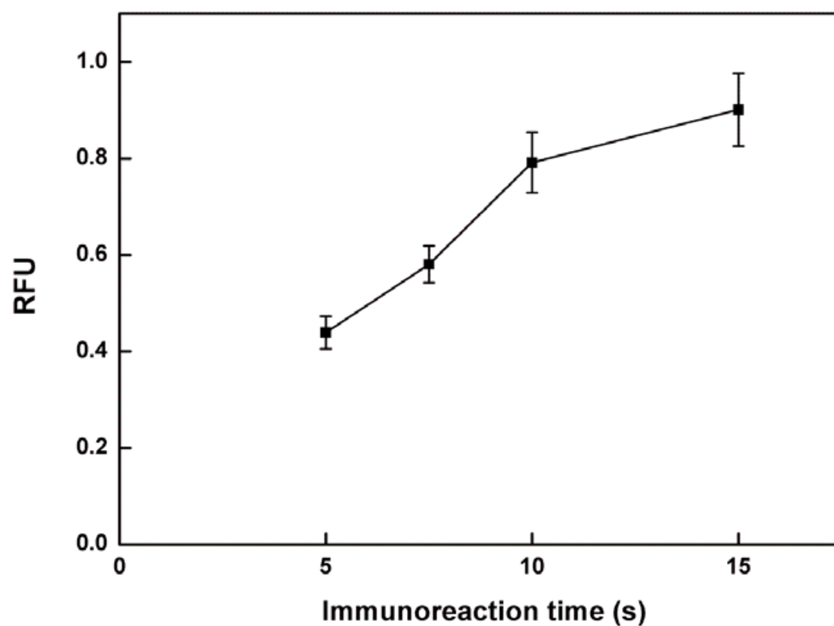

Figure 4. Immunoreaction time versus the peak height of CRP (10 mg/L). Electrophoresis conditions: run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm. Voltages were programmed as shown in Table 1. Error bars represent the standard deviations (n = 4) and the response is in relative fluorescence units (RFU).

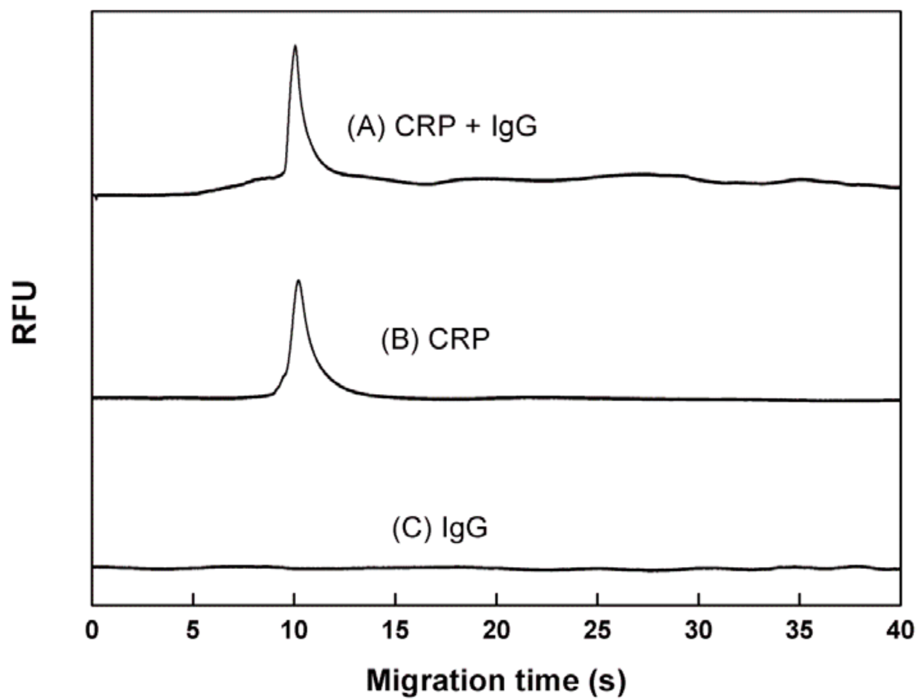

Figure 5. Electropherograms of CRP and a control, IgG. (A) On-chip immunoassay of 85 nM CRP and 85 nM IgG. (B) On-chip immunoassay of 85 nM CRP (10 mg/L). (C) On-chip immunoassay of 85 nM IgG. Electrophoresis conditions: run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm. Voltages were programmed as shown in Table 1.

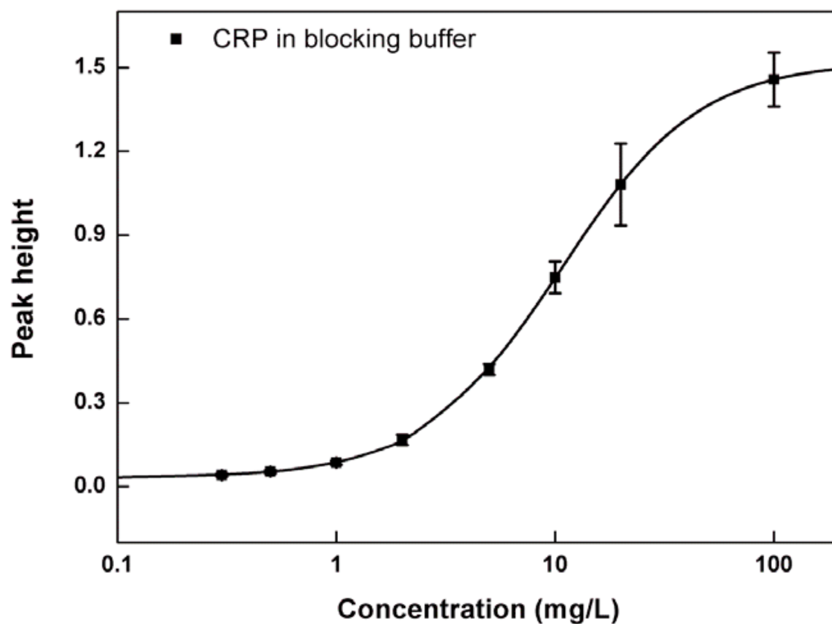

Figure 6. Calibration curve of the CRP concentration in the blocking buffer versus the peak height. Square spots with the bar show the dynamic range (0.3–100 mg/L) of CRP (n = 8) in blocking buffer. Electrophoresis conditions: run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm. Voltages were programmed as shown in Table 1. Error bars represent the standard deviations (n = 4) and the response is in relative fluorescence units (RFU).

3.3 On-chip sandwich immunoassays in human serum samples

To verify the applicability of the proposed on-chip sandwich immunoassay to a real sample, CRP samples in human serum were analyzed. CRP was spiked into a 10-fold diluted CRP-free human serum with a blocking buffer. This was used to prevent long immunoreaction times and the clogging of the microchannels due to the high-viscosity serum matrix. As shown in Fig. 7, the results obtained with the samples of 0.3–20 mg/L CRP in serum (n = 4) were in good agreement with those from CRP samples in the blocking buffer. The parameter values estimated from fitting to the data were as follows: Min = 0.033 ± 0.001, Max = 1.279 ± 0.010, EC_{50} = 8.940 ± 0.105, B = –1.508 ± 0.016 and r^2 = 0.999 (n = 7). As shown in Fig. 8, the migration time of CRP in the diluted serum was also equal to that of CRP in the blocking buffer. In spite of the poor reproducibility and resolution obtained with a PT microchip, the effect of the diluted serum matrix did not significantly interfere with the sandwich immunoassay. Moreover, our PT microchip immunoassay provided a sensitivity level that was sufficient for the clinical cut-off level (10 mg/L) and is thus feasible for use as a point-of-care device for risk screening for CRP-related diseases.

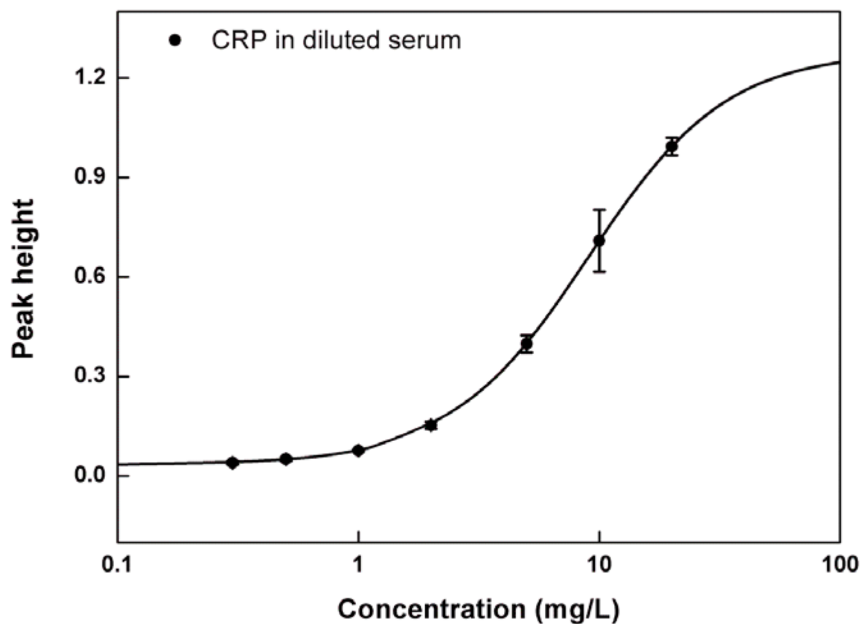

Figure 7. Calibration curve of spiked CRP in serum versus the peak height. Circle spots with the bar represent the 0.3, 1, 2, 5, 10 and 20 mg/L CRP concentrations (*n* = 7) spiked in serum (1:10 diluted). Electrophoresis conditions: run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm. Voltages were programmed as shown in Table 1. Error bars represent the standard deviations (*n* = 4) and the response is in relative fluorescence units (RFU).

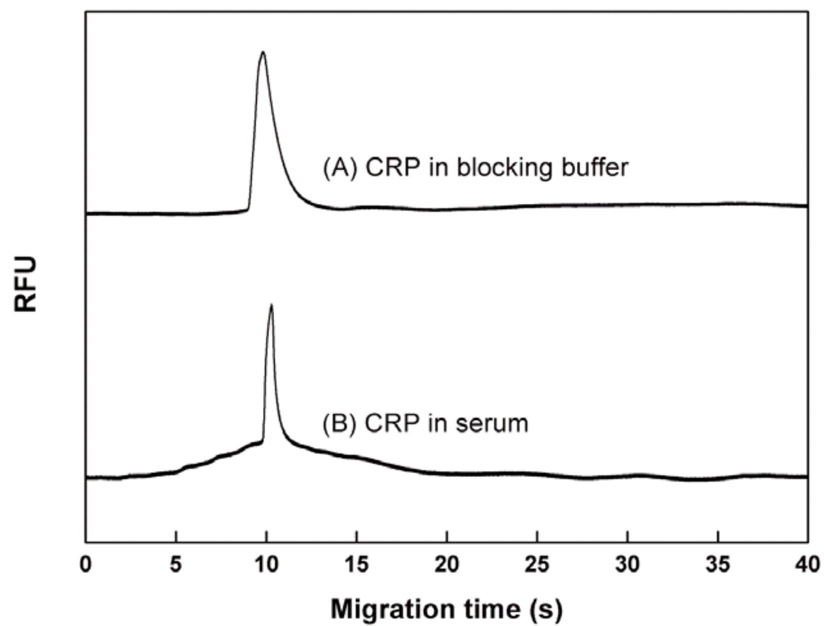

Figure 8. Electropherograms: (A) CRP (10 mg/L) in the blocking buffer and (B) spiked CRP (10 mg/L) in serum versus the peak height. Electrophoresis conditions: run buffer, 20 mM sodium tetraborate; LIF detection, λ_{ex} = 488 nm, λ_{em} = 520 nm. Voltages were programmed as shown in Table 1.

4. Conclusions

A PT microchip capable of being used with microfluidic sandwich immunoassays was developed. In order to overcome the problem of the low separation efficiency of a PT microchip as compared to a glass or polymer microchip, CTI was applied. After immobilizing CRP with the capture antibody on the surface of a microbead, the detection antibody was conjugated with the CRP. The fluorescent tag cleaved from the detection antibody was injected into the separation channel for electrophoresis with LIF detection. The microfluidic immunoassay using a direct printing process has the advantages of completing microchip fabrication and immunoassay within 15 and 35 min, respectively. With this on-chip immunoassay, the cardiac marker CRP in serum could be detected in the concentration range of 0.3 to 20 mg/L, demonstrating its potential as a point-of-care device in clinical diagnosis.

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국문초록

폴리에스터-토너 마이크로 유체칩을 이용하여 C 반응성 단백질을 검출하기 위해 칩 상에서 면역분석법을 수행하였다. C 반응성 단백질는 급성염증 시 증가 정도로 질병의 초기 증상을 진단할 수 있어 임상 목적으로 사용되고 있는 특이 혈청 단백질이며, 특이적 결합능을 가진 면역분석법으로 많은 연구가 진행되어 왔다. 최근 직접 인쇄 공정을 통해 일회용 폴리에스터-토너 마이크로칩을 손쉽게 제작하는 기술이 개발되었으며, 다양한 연구 분야에 응용되고 있다. 그러나 폴리에스터-토너 마이크로칩은 낮은 분리 효율을 갖는 단점이다. 이를 극복하기 위해 마이크로비드를 사용하여 표면에 고정되는 포획 항체의 양을 늘리고, 검출 항체 대신에 항체에 붙어 있는 표지만 마이크로채널로 주입되는 분리 가능한 표지를 이용하였다. 폴리에스터-토너 마이크로칩에서의 전체 분석 시간은 35분 이내이다. 10배 묽힌 혈청에 있는 C 반응성 단백질의 동적영역은 0.3에서 20mg/L이며, 검출한계는 약 0.3mg/L이다. 이러한 연구는 임상시험 시 혈청에 있는 C 반응성 단백질의 정량분석 가능성을 입증해 준다.

주요어: 폴리에스터-토너 마이크로칩, 마이크로 유체 면역분석법, C 반응성 단백질, 분리 가능 표지 면역분석법

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