An integrated microfluidic chip for immunocapture, preconcentration and separation of β -amyloid peptides

Reza M. Mohamadi,¹ Zuzana Svobodova,² Zuzana Bilkova,² Markus Otto,³ Myriam Taverna,⁴ Stephanie Descroix,¹ and Jean-Louis Viovy^{1,a)}

¹Curie Institute/CNRS/Université Pierre et Marie Curie, UMR 168, Paris, France

²Department of Biological and Biochemical Sciences, University of Pardubice, 53210 Pardubice, Czech Republic

³Department of Neurology, University of Ulm, Steinhövelstrasse 1, 89075 Ulm, Germany

⁴Faculté de Pharmacie, Institut Galien Paris Sud, University of Paris Sud, UMR 8612, Chatenay Malabry, France

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We present an integrated microfluidic chip for detection of β -amyloid ($A\beta$) peptides. $A\beta$ peptides are major biomarkers for the diagnosis of Alzheimer's disease (AD) in its early stages. This microfluidic device consists of three main parts: (1) An immunocapture microcolumn based on self-assembled magnetic beads coated with antibodies specific to $A\beta$ peptides, (2) a nano-porous membrane made of photopolymerized hydrogel for preconcentration, and (3) a microchip electrophoresis (MCE) channel with fluorescent detection. Sub-milliliter sample volume is either mixed off-chip with antibody coated magnetic beads and injected into the device or is injected into an already self-assembled column of magnetic beads in the microchannel. The captured peptides on the beads are then electrokinetically eluted and re-concentrated onto the nano-membrane in a few nano-liters. By integrating the nano-membrane, total assay time was reduced and also off-chip re-concentration or buffer exchange steps were not needed. Finally, the concentrated peptides in the chip are separated by electrophoresis in a polymer-based matrix. The device was applied to the capture and MCE analysis of differently truncated peptides $A\beta$ (1–37, 1–39, 1–40, and 1–42) and was able to detect as low as 25 ng of synthetic $A\beta$ peptides spiked in undiluted cerebrospinal fluid (CSF). The device was also tested with CSF samples from healthy donors. CSF samples were fluorescently labelled and pre-mixed with the magnetic beads and injected into the device. The results indicated that $A\beta$ 1-40, an important biomarker for distinguishing patients with frontotemporal lobe dementia from controls and AD patients, was detectable. Although the sensitivity of this device is not yet enough to detect all $A\beta$ subtypes in CSF, this is the first report on an integrated or semi-integrated device for capturing and analyzing of differently truncated $A\beta$ peptides. The method is less demanding and faster than the conventional Western blotting method currently used for research. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4931394>]

I. INTRODUCTION

Alzheimer's disease (AD) is one of the most common age-related neurodegenerative disorders, yet diagnosis of AD is still mainly based on clinical and neurological symptoms. Debates regarding the most relevant AD specific biomarkers are ongoing; however, there is a strong consensus on the fact that the accumulation of β -amyloid ($A\beta$) peptides in amyloid plaques is one of the hallmarks of the progression of the disease.^{1–5} This accumulation is

^{a)} Author to whom correspondence should be addressed. Electronic mail: Jean-Louis.Viovy@curie.fr. Tel./Fax: +33 1 40 51 06 36.

associated with a modification in the balance between differently truncated subtypes of A β peptides. A typical concentration of A β peptides in cerebrospinal fluid (CSF) is normally between 1 and 10 ng/ml. A β peptides can also be found, with 10 to 100 times lower concentrations than CSF, in urine and blood. This makes A β peptides potential targets for non-invasive monitoring of AD.^{6–10} The ratios of concentrations of differently truncated A β peptides are relatively stable in CSF samples of healthy individuals; in contrast, a selective reduction of A β 1–42 in CSF from AD patients has been reported.^{11,12} A β 1–40 was also reported to be an important biomarker for distinguishing patients with frontotemporal lobe dementia (FTLD) from controls and AD patients.^{13–15} An enzyme-linked immunosorbent assay (ELISA) based method for detection of A β 1–40 and A β 1–42 peptides is currently available.¹⁶ Although very promising, ELISA requires specific antibodies and developing antibodies capable of differentiating all the differently truncated A β peptides (A β 1–37, A β 1–39, A β 1–40, and A β 1–42), which are different only by one or two amino acids, is challenging and cross reaction of specific antibodies with very similar differently truncated A β peptides can lead to false positive results. Alternate methods using Mass Spectrometry as an end-point detection means, such as LC-MS, can unambiguously differentiate differently truncated peptides, but they unfortunately suffer from other drawbacks. First, the sensitivity of MS limits its use for trace analysis, and due to unpredictable differences in ionization efficiencies, it is not a quantitative method. Since A β -based diagnosis is primarily based on concentration differences, this limitation is a major drawback. It could in principle be overcome by isotopic labelling, but this method is not suitable for clinical samples in which the two species to compare are contained in the same sample.

Western blotting method, in which size-based separation of the A β peptides is followed by staining using a generic antibody which can react to the common part of the peptides, has also been applied in the analysis of A β peptides; however, this method is labor intensive, hard to automate, and is limited to research laboratories.

Microfluidic devices have shown many advantages for the analysis of protein and peptide biomarkers in biological fluids such as CSF, serum, or urine.¹⁷ Detection and quantification of low abundance biomarkers in complex biological media often requires selective on-chip enrichment of the target molecules in an immunocapture step. Early attempts at immunocapture in microfluidic devices were conducted by grafting antibodies on microchannel walls, on packed bed of beads, or on monoliths polymerized directly in the microchannel.^{18–22} Application of self-assembled magnetic beads pre-coated with antibodies in microfluidics offers additional advantages: it does not require in-chip chemistry, and magnetic beads can be magnetically manipulated using permanent magnets or electromagnets, independently of microfluidic or biological processes.^{23–28}

We previously proposed microfluidic devices involving self-assembled superparamagnetic beads in a geometry optimizing permeation and interactions between particles and fluid. These devices were successfully applied to on-chip enzymatic digestion of proteins and also to immunocapture and off-chip analysis of A β .^{29–31} We also developed microchip electrophoresis (MCE) methods for the analysis of differently truncated A β peptides.^{32–34} The off-chip analysis methods, however, required sample manipulation by pipetting and extra re-concentration of the eluted sample using a speedvac, which was time consuming, increased the risk of cross-contamination, and also lowered run to run reproducibility.

Here, we introduce an integrated chip, in which three analytical steps for detection of differently truncated A β peptides including immunocapture, elution, and preconcentration, and electrophoresis separation are combined (Figure 1). In this device, samples were injected hydrodynamically, allowing us to inject large volumes of sample and benefit from a magnetic bead plug to increase the capture efficiency. Elution step was performed by electroelution, taking advantage of the plug flow profile to minimize dispersion and to elute the captured sample in a minimal volume. To further improve the sensitivity of detection, the eluted sample was re-concentrated on a hydrogel membrane prepared by *in situ* photopolymerization of Poly(EthyleneGlycol) diacrylate (PEG-DA). PEG-DA materials are highly hydrophilic and rather inert in term of protein adsorption. By adapting the prepolymer composition, the pore size in PEG-DA membrane can be easily tuned down to a few nanometers, needed for the

FIG. 1. Schematic design of the integrated chip. (a) CSF sample was labeled with the fluorescent dye off-chip, mixed and incubated with magnetic microbeads, and then injected into the integrated microchip. Schematic layout of the integrated microfluidic device is explained. The integrated chip includes three compartments: (I) a plug of self-assembled magnetic beads coated with antibody inside the microchannel for immunocapture of the target peptide ($A\beta$), (II) a photopolymerized hydrogel plug for preconcentration of the eluted sample, and (III) a MCE unit coupled with fluorescence detection (reservoirs 1 to 4 were used for applying voltage during preconcentration and electrophoresis steps). (b) In step 1, using a syringe pump, sample was injected to the chip from the inlet, and the target peptide was captured on the antibody coated magnetic beads. In step 2, by applying voltage, target was eluted from the beads and concentrated on the photo-polymerized membrane. In step 3, the concentrated target was injected to the electrophoresis channel.

preconcentration of small peptides.^{35,36} Compared to the off-chip analysis method,³² integration of this nano-membrane in the new chip eliminates time consuming re-concentration and buffer exchange steps, which required off-chip operation with a speedvac concentrator equipment. The eluted and re-concentrated peptides on the nano-membrane are then separated by detected MCE and fluorescent detection.

II. MATERIAL AND METHODS

A. Reagents, polymer, and sample preparation

$A\beta$ peptides ($A\beta$ 1-37, $A\beta$ 1-39, $A\beta$ 1-40, and $A\beta$ 1-42) were purchased from AnaSpec (Fremont, CA). $A\beta$ peptides were dissolved in an alkaline buffer provided by the supplier and labelled with Fluoprobe-488 NHS (Interchim, Montluçon, France) prepared in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml. In general, a 10 fold excess of the fluorescent dye was used for labelling. When working with lower concentrations of the peptides (100 ng/ml and less), a 50 fold excess of the fluorescent dye was used for labeling. The mixtures were then incubated at 4 °C for 1 h in the dark. Methylcellulose (MC-400) was purchased from Sigma

Chemical Co. (St. Louis, MO). The methylcellulose solutions (0.2% or 2%) were prepared according to the manufacturer's suggestions in the appropriate electrophoresis buffer for each sample. For the fluorescent labelled IgG, we used 0.2% methylcellulose in Tris-HCl 20 mM, pH 8.3, while for A β peptides, we used 2% methylcellulose in Borate 40 mM, pH 9.5. Mouse IgG1 labelled with Alexa Fluor-488 were purchased from Invitrogen (CA).

B. Preparation of the magnetic beads

For capturing of A β in buffer and CSF, we used 2.8 μ m M280 Dynabeads coated with sheep anti-mouse IgG (Dyna, Oslo, Norway) activated with monoclonal antibody anti-A β 6E10 (Covance, Emeryville, CA). To activate the beads, 80 μ g of monoclonal antibody anti-A β 6E10 (Covance, Emeryville, CA) were added to 2 ml of Dynabeads (10 mg/ml, 6.7×10^8 beads/ml). The activated beads were kept in 4 °C. For each capture experiment, 0.1 mg of the antibody coated microparticles was washed and suspended in 50 μ l TBST buffer (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, and 0.05% Tween-20). In the preliminary tests, we used commercial magnetic particles coated with anti-IgG (SiMAG-Goat anti-mouse IgG 1 μ m from Chemicell, Germany). These beads were used to capture fluorescently labeled IgG.

C. Preparation of CSF samples

The CSF samples analyzed in this study were obtained from patients attending the outpatient Memory Clinic in Ulm. Collection and analysis of the CSF sample were approved by the Ethics Committee in Ulm. The obtained CSF was aliquoted within 2 h and stored at -80 °C. CSF was directly labeled by adding 10 μ l of Fluoprobe NHS-488 (10 mg/ml in DMSO) to 50 μ l or 250 μ l of CSF. The mixture was incubated at 4 °C for 1 h in the dark. The labelled CSF was then mixed with the same volume of radio immunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl pH 7.2, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM Na₃VO₄, 1 mM PMSF, 1 mM EDTA, and 1 mM protease inhibitor).

D. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry of immunocaptured A β peptides

For MALDI-TOF mass analysis, CSF samples were incubated with the anti-A β coated magnetic beads, overnight. The beads were then suspended in 200 μ l of 0.16% NH₄OH at room temperature for 10 min under sonication. The eluted A β peptides were separated from the beads and dried out in a speedvac. Samples were re-dissolved in a solution of 60% acetonitrile and 0.1% trifluoroacetic acid. An aliquot of 0.5 μ l was mixed with 0.5 μ l saturated α -cyano-hydroxycinnamic acid, 50% acetonitrile, 0.1% trifluoroacetic acid and was spotted onto a MALDI plate and allowed to dry. All spectra were acquired with a 4800 plus TM MALDI-TOF-TOF mass spectrometer (Applied Biosystems, Foster City, CA).

E. Fabrication of the integrated microfluidic chip

Microchips were fabricated by rapid prototyping and Polydimethylsiloxane (PDMS) technology,³⁷ starting with a master composed of a positive relief of SU-8 resin on a silicon wafer (Neyco, France). A Plexiglas (Polymethyl Methacrylate) mold containing plastic relief was used, which precisely define the size and location of the magnets. A PDMS replica of the master was formed, while the Plexiglas mold was fixed on top of the silicon wafer. The PDMS was then peeled away and holes were pierced for tubing connection and for the reservoirs. The replica was permanently sealed with a PDMS-coated glass slide. Bonding was enhanced and made irreversible by oxidizing both the replica and the cover in a plasma discharge for 1 min prior to bonding. Silicone tubing was then added at the inlet and the outlet of the channels. The channel cross section in the immunocapture part was 1 mm (width) by 50 μ m (depth) and the channel length was 2 cm. In the MCE part, the channel cross section was 100 μ m (width) by 50 μ m (depth), and the effective separation length was 35 mm (Figure 1).

After the preparation of the PDMS chips, a hydrogel membrane was fabricated within the microchannel in a specified area inside each chip (Figure 1). A photomask containing the membrane shape was designed in AUTOCAD 2006 and printed using a 24 000 dpi high-resolution printer at Selba S.A. (Versoix, Switzerland). The mask was then inserted into the field-stop plane of a microscope (Zeiss Axiovert 200, tube lens magnification 4 $\times$, objective 10 $\times$, Zeiss Achroplan, 10 $\times$ /0.25). A 100 W HBO mercury lamp served as the source of UV light. A filter set allowing wide UV excitation (Excitation 365HT25, emission 400EFLP, Omega Optical, VT) was used to select light of the desired wavelength. A VS25 shutter system (Uniblitz), driven by a computer-controlled D122 shutter driver, was used to provide pulses of UV light with the specified duration (200 ms). The shutter was controlled by an in-house software (written in LabView, National Instruments, Austin, TX). To photopolymerize the membrane, we prepared a solution of 5% (w/w) of Darocur 1173 (Sigma Aldrich) initiator in 65% (w/w) poly(ethylene glycol)(400) diacrylate (PEGDA, Polysciences) and 30% (w/w) ddH₂O. This solution was then driven through the microfluidic device with a Kd Scientific 210 P syringe pump. Then, the UV light passing through the photomask was projected in the specified area in the microchannel for 200 ms. Unpolymerized precursor solution was then flushed from the microchannels using ddH₂O.

F. Microchip surface coating and measurement of electroosmotic flow (EOF)

To control EOF in the PDMS channels, we used a solution of 0.2% of a homemade copolymer PDMA-AGE (kindly provided by M. Chiari's group, CNR, Milano) in ddH₂O, which was introduced into the microchannels.³⁸ The chip was kept in the dark for at least 30 min, and the excess polymer was rinsed out by extensive ddH₂O washing. We used current monitoring method to measure EOF in the PDMS channel.³⁹ To control nonspecific adsorption of the sample into the microchannel, 0.01% Tween-20 was also added to the electrophoresis buffers.

Before each experiment, two rare earth permanent magnets (Neodymium Iron Boron, Hangzhou, China) were inserted into cut-outs in the PDMS-block enclosing the microchannels (Figure 1). Both magnets have dimensions of 4 mm $\times$ 4 mm $\times$ 16 mm, with a magnetic remanence of 1.3 T at the pole and a polarisation in the longest dimension. The magnets point in the same orientation with regards to the channel axis, and make an angle of 20° with the channel.

The electrophoresis channel was then filled with a solution of methylcellulose as the separation medium. The MC solution was introduced from reservoir 2 (Figure 1) while the reservoirs 1 and 3 were opened, and the other reservoirs were kept closed. The beads' suspension was then injected into the microchannel at a flow rate of 5 μ l/min. When approaching the magnet zone, beads assembled into columns, oriented in the direction of the flow and remain trapped between the two magnets. The plug was then rinsed with TBST buffer at the same flow rate.

G. Microchip operation, sample injection, and washing

The sample introduction into the immunocapture column and the following washing steps were performed either hydrodynamically using a syringe pump (at flow rates ranging from 0.5 to 4 μ l/min) or electrokinetically by applying an electric field (Figure 1). After sample introduction, the unbounded antigens were washed away by introducing TBST buffer at flow rate of 4 μ l/min for 10 min. The captured antigens were then eluted by applying electric potential between the outlet reservoir and the preconcentration membrane (reservoir 4, Figure 1). This way, the eluted sample was reconcentrated on the surface of the membrane. A HVS448 3000 V power supply (LabSmith, Livermore, CA), controlled by a PC, was used for electroelution and for MCE afterwards. Different elution buffers such as 100 mM SDS in Tris-HCl, 200 mM Glycine, pH 2, and 0.16% NH₄OH in H₂O were tested.

The preconcentrated peptides were then injected into the electrophoresis channel by the pinched injection method⁴⁰ for 30 s. The applied voltages are summarized in Table I. An inverted fluorescence microscope, Olympus IX71, equipped with a 10 $\times$ 0.3 NA objective lens (Olympus, Tokyo, Japan) was used to detect, in real time, fluorescently labelled samples. Illumination was provided by a 100-W mercury arc lamp. A Nikon digital DS-Qi1 camera (Nikon, Tokyo, Japan) was used to capture images of the detection zone. Still images were

TABLE I. Applied voltages to each reservoir (Figure 1) during elution and electrophoresis of captured samples (NC = Not connected).

Step	Time (s)	Reservoir (1) (V)	Reservoir (2)	Reservoir (3)	Reservoir (4)	Reservoir (Outlet)
1-Elution	240	1500	NC	NC	NC	0 V
2-Injection	25	0	NC	+1500 V	NC	NC
3-Separation	150	+750	0 V	+750 V	+1500 V	NC

recorded at a frequency of 10 images per second, and then, the intensity from the selected detection zone was integrated using an image processing software (NIS Elements by Nikon). The intensity corresponding to each frame was recorded in an electropherogram for each separation.

III. RESULTS AND DISCUSSION

A. Validations and optimization of different steps in the integrated chip

The analytical workflow in the integrated chip involves three main steps: sample injection and washing to remove interfering compounds, immunoextraction and electrokinetic preconcentration of the eluted peptides or proteins in a small volume (nanoliters), and an electrophoretic separation of the captured peptides or proteins. The sample introduction and washing steps could be performed either electrokinetically or hydrodynamically. We compared the efficiency of immunocapture with electrokinetic sample injection versus hydrodynamic sample injection using a model protein (Fluorescent IgG). With a similar injection time, the intensity of the eluted peak was at least 10 times lower than that obtained with hydrodynamic sample injection (Figure 2(a)). We have observed a similar trend in our previous report where monolithic stationary phase was applied to nonspecific preconcentration of polyaromatic hydrocarbons.⁴¹

Electrokinetic sample injection allows easier integration with the downstream electrokinetic concentration and separation and can limit sample dispersion; however, it limits the amount of injected samples, and it is also limited by the ionic charge of the target and the immunosupport. If applied for a long period of time, electrokinetic sample injection can also cause Joule heating. Hydrodynamic injection, however, can allow injection of large sample volume, but due to high back pressures, it has never been applied to other on-chip immunocapture methods based on *in situ* polymerized monoliths, packed beds, or gels. Unlike the above mentioned methods and as a result of its dynamic and "self-healing" properties, magnetic column allows hydrodynamic injection of large volumes at flow rate in the range of μL per minute with negligible back pressure. One important factor that we had to consider here was the resistance of the magnetic plug under the flow. We evaluated the stability of the magnetic plug in a channel with $50\text{ }\mu\text{m}$ depth and 1 mm width. Microscopy monitoring showed that the magnetic plug in this channel was stable under flow rates up to $4\text{ }\mu\text{L}/\text{min}$, corresponding to a linear speed of $1300\text{ }\mu\text{m s}^{-1}$, while higher flow rates created small fractures in the plug. These fractures were deleterious towards capture efficiency and reproducibility as the liquid mainly flowed through the fractures instead of flowing homogeneously through the whole plug.

Once the capture step was done, we had to elute the captured peptides from the magnetic column. To achieve an efficient and controlled electro-elution of the captured targets, we also need to suppress the EOF in the microchannels. Electrokinetic migration in uncoated PDMS channels usually yields unstable and high EOF, and high EOF acts as a counter flow to the elution. We measured EOF in the PDMS channel with and without a magnetic plug. EOF in uncoated PDMS (in 20 mM Tris-HCl pH 7.5) was $6 \times 10^{-4}\text{ cm}^2\text{ v}^{-1}\text{ s}^{-1}$ and in the channel with magnetic plug was $3 \times 10^{-4}\text{ cm}^2\text{ v}^{-1}\text{ s}^{-1}$ (Figure 2(b)). The high EOF in the uncoated channels created turbulent instability of the magnetic plug (supplementary video S.1).⁴⁴ After coating with PDMA-AGE, the EOF of the PDMS channel was reduced by two orders of magnitude, and the elution of captured targets was successfully achieved (supplementary video S.2).⁴⁴

FIG. 2. (a) Validation of the performance of different compartments of the integrated chip. (a) Electropherogram of Alexa labelled IgG captured in the integrated device by electrokinetically (lower trace) sample injection versus hydrodynamic sample injection (top trace). Original concentration of sample was 30 nM. (b) EOF measurements in uncoated PDMS channel with and without magnetic plug. The error bar shows standard deviations ($n = 5$). After coating with PDMA-AGE, the EOF was $1 \times 10^{-6} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, two order of magnitude less than uncoated chip. EOF measurements were done in 20 mM Tris-HCl buffer pH 7.5. (c) Preconcentration of fluorescent dye Fluoprobe-488 NHS (30 pM) on the photopolymerized membrane which can be seen in the inner box in this graph. Fluorescent intensity on the membrane is plotted against time (the scale bar is 100 μm). (d) Comparison of two electropherograms of original concentration of fluorescent dye (dashed) and after 5 min preconcentration (solid) (the fluorescent dye appears as two separated peaks which can be two different forms of the dye).

In the previous report,³² the elution was done by flowing an elution buffer in the chip, in the integrated chip the elution step was done electrokinetically by applying voltage and therefore controlling the EOF is crucial in this chip.

Electroelution of the captured peptides takes a few minutes. During this period, the eluted sample diffuses in the channel, and band broadening occurs, which will be deleterious to achieve a high sensitivity in the electrophoresis channel. We thus designed and integrated a nano-membrane in our chip to concentrate the eluted sample. We fabricated a hydrogel membrane by *in situ* photopolymerization of PEG-DA. In stop-flow lithography, a thin unpolymerized layer remains between the PEG-DA polymer and the channel's walls due to PDMS permeability and interference of oxygen with polymerization.²⁷ Unattached membrane could be detrimental in our experiments, allowing for leakage of the proteins or peptides of interest out of the concentration domain. We counteracted this problem by polymerizing the membrane in a less hydrophilic solution (30% aqueous buffer) and exchanging the initial solution with an aqueous buffer during the washing step. During this process, the polymerized hydrogel membrane adsorbs water and swell,^{42,43} We measured the change in volume of photopolymerized hydrogel (in an open microchannel, data not shown). This swelling corresponded to 20% of the initial volume. We tested the membrane for preconcentration of a fluorescent dye (981 Da) (30

pM), and after 5 min of preconcentration, a saturation of the detection camera was achieved and the intensity curve reached a plateau (Figures 2(c) and 2(d)).

The whole integrated process was then tested using the model protein (Alexa labelled IgG) and magnetic beads coated with anti-IgG. The sample was hydrodynamically introduced using a syringe pump (100 μ l at 1 μ l/min) while the elution and preconcentration on the PEG-DA membrane were carried out using an electric field. In these conditions, we were able to detect 10 ng Alexa-IgG spiked in 100 μ l buffer. Such concentration was not detectable by direct electrophoresis of the original sample. The electrophoresis was done in a solution of methylcellulose. Conditions for optimal separation of the A β peptides were derived from the previously reported ones.^{32,34} In order to avoid possible complications caused by the sample preparation step, the validation steps were done using a standard fluorescent protein (IgG), and the results show that the integration of the three compartments was successfully achieved. We have shown (Figure 2(b)) that the EOF in PDMA-AGE coated channel is suppressed, the elution and preconcentration on PEG-DA nano-membrane are optimized and the eluted sample was successfully transferred to the electrophoresis channel.

B. Immunocapture and electrophoresis of synthetic A β peptides spiked in human CSF

After preliminary validations, we applied our system to immunocapture and MCE analysis of differently truncated synthetic A β peptides. Figures 3(a) and 3(b) show the electropherogram of 3 standard A β peptides (A β 1-39, A β 1-40, and A β 1-42) spiked in human CSF. The electropherograms were achieved after performing the complete on-chip analytical steps: immunocapture, electroelution, electrokinetic preconcentration, and separation. The whole process for analysis of these A β peptides was done in 3 h while the same analysis using conventional Western blotting requires about 36 h. The efficiency of capture was also tested using different concentration of A β 1-37 and A β 1-42 spiked in CSF (Figure 3(c)). The results showed that we are able to detect a 25 ng labeled A β peptides spiked in 100 μ l CSF. The peak for the fluorescent dye in these electropherograms is serving as an internal standard to correct possible variations of the retention times of the peaks for A β peptides. While we are able to detect a, namely, concentration of 25 ng A β peptide spiked in 100 μ l CSF, but due to the difficulty of solubilizing synthetic A β peptides, it is not possible to calculate the actual concentration of the soluble peptides and therefore the exact limit of detection.

FIG. 3. The integrated was tested with synthetic A β peptides spiked in CSF, all the steps Immunocapture, elution, preconcentration, and MCE were done on chip. (a) Electropherogram from a labelled A β 1-40 spiked in CSF, (b) Mixture of 3 peptides A β 1-39, A β 1-40, and A β 1-42 at 2.5 μ g/ml in the original sample was captured and analyzed (detection was done at 30 mm from the cross section). MCE was done in 2% MC in 40 mM borate buffer pH 9.5. A β * corresponds to the peak for aggregated or modified peptides. (c) Different amounts of A β 1-37 and A β 1-42 were spiked in 100 μ l CSF.

Using a previously described western blotting method,³³ we tested the waste and the eluted fraction for the trace of peptides, which may have not been captured on the column, and the results showed that >90% of the peptide were captured on the beads; however, the total recovery after elution was >60%, indicating that some of the peptides were not eluted from beads. We also compared the capture efficiency of the labeled and unlabeled peptides and similar band intensities were achieved, showing that fluorescent labeling did not interfere with antibody-peptide interaction.

C. Immunocapture and MCE of A β peptides in human CSF

Finally, we demonstrated the possibility to apply our integrated microsystem for the detection of A β peptides in human CSF. Considering the sensitivity obtained for synthetic A β peptides (25 ng) and the expected physiological concentrations (1–10 ng/ml), one could expect that we will need at least 2 to 3 ml of CSF to be able to detect A β peptides. Indeed, when using 50 μ l of labelled CSF and with the flow rate optimal for high capture efficiency, we could not detect any A β peptides in the electropherogram (Figure 4(a)). We then developed an alternate protocol, in which similar to the current Western blotting methods, the capture step was performed off-chip and overnight in 10 times higher volume of CSF (500 μ l CSF) using magnetic beads activated by anti-A β mAb. After washing, the magnetic beads were injected to the microchip, and then the elution, re-concentration and electrophoresis steps were performed on chip. The electropherogram in Figure 4(a) shows that, using this method, we could detect A β 1-40 in human CSF. The peak identity was confirmed by injecting a blank sample and also co-injection of fluorescent dye and also by off-chip MALDI-TOF mass analysis on the eluted samples (Figure 4(b)). The concentration of A β 1-40 is 10 times higher than that of other A β peptides, and A β 1-38 generally generates high signal in MALDI-TOF mass analysis.¹¹ We repeated 3 to 5 times the same test on two independent CSF samples. In all of the tests, peak for A β 1-40 was detectable, yet quantitative reproducibility of the test needs to be improved.

IV. CONCLUSIONS AND OUTLOOK

In conclusion, we developed an integrated microfluidic system in which immunocapture using self-assembled magnetic beads was integrated with MCE. To improve the sensitivity of detection, we also integrated a preconcentration site using photopolymerized hydrogel. In this

FIG. 4. The integrated chip was tested with fluorescently labeled CSF. (a) Representative electropherogram of A β peptides in two human CSF samples after immunocapture in the integrated chip are presented. The testes were done in 50 μ l (CSF-1*) and 500 μ l CSF (CSF-1 and CSF-2) (elution and MCE condition were similar to Figure 3). (b) Off-chip MALDI-TOF mass spectrum of eluted A β peptides after immunocapture from same human CSF sample as in part (a) (CSF-2).

microfluidic system, sample injection to the magnetic column was achieved hydrodynamically; this imposed a “stirred bed” behavior to the capture column, increasing the efficiency of capture and the flow throughput. The elution was achieved electrokinetically, and the eluted sample was re-concentrated on a nano-porous membrane. This step replaced a time consuming manual re-concentration step in the not-integrated chip. The device has the potential for automated immunocapture and electrophoretic analysis of different proteins and peptides, using commercially available antibody coated magnetic beads. We tested our microchip for the analysis of A β peptides in human CSF. We were able to capture more than 90% of the injected peptide, to elute more than 60% of the captured peptides in 5 min, and to detect as low as 25 ng of labeled A β peptides spiked in 100 μ l of CSF. The operation of this device is faster, less demanding in terms of manpower and reagents than conventional Western blotting method. It would thus be very interesting for routine clinical analysis of A β in CSF. The device also has the advantage of using a single antibody for differentiating differently truncated A β peptides, which is not possible in EILSA methods. These peptides are recognized as biomarkers at an early stage of Alzheimer’s disease. For this application, an increase in sensitivity by a factor between 5 and 10 is still needed. We believe, however, that such progress is within reasonable reach with subsequent engineering and optimization regarding the balance of sizes and geometries between the capture, re-concentration, and separation components.

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