A pump-free microfluidic device for fast magnetic labeling of ischemic stroke biomarkers

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Abstract
This research proposes a low-cost and simple operation microfluidic chip to enhance the magnetic labeling efficiency of two ischemic stroke biomarkers: cellular fibronectin (c-Fn) and matrix metallopeptidase 9 (MMP9). This fully portable and pump-free microfluidic chip is operated based on capillary attractions without any external power source and battery. It uses an integrated cellulose sponge to absorb the samples. At the same time, a magnetic field is aligned to hold the target labeled by the magnetic nanoparticles (MNPs) in the pre-concentrated chamber. By using this approach, the specific targets are labeled from the beginning of the sampling process without preliminary sample purification. The proposed study enhanced the labeling efficiency from 1 h to 15 min. The dynamic interactions occur in the serpentine channel, while the crescent formation of MNPs in the pre-concentrated chamber, acting as a magnetic filter, improves the biomarker-MNP interaction. The labeling optimization by the proposed device influences the dynamic range by optimizing the MNP ratio to fit the linear range across the clinical cutoff value. The limits of detection (LODs) of 2.8 ng/mL and 54.6 ng/mL of c-Fn measurement were achieved for undiluted and four times dilutions of MNP, respectively. While for MMP9, the LODs were 11.5 ng/mL for undiluted functionalized MNP and 132 ng/mL for four times dilutions of functionalized MNP. The results highlight the potential use of this device for clinical sample preparation and specific magnetic target labeling. When combined with a detection system, it could also be used as an integrated component of a point-of-care platform.

Keywords Magnetic nanoparticle · Microfluidic · Sample preparation · Fibronectin · MMP9 · Magnetoresistive

Introduction
Cerebrovascular accidents or stroke are a major disease-causing death worldwide in the last two decades after the ischemic heart attack [1]. Among the stroke cases, ischemic stroke type is responsible for up to 71% of cases globally [2]. Ischemic stroke can be defined as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction [3]. The leading cause is a blood clot that blocks blood circulation to the brain. In acute ischemic...
stroke cases, the advanced treatment is recombinant tissue plasminogen activator (rt-PA), by intravenously drug delivery to dissolve the blood clot (< 4.5 h) after the onset of the symptoms [2, 4]. Despite the fact that around a quarter of ischemic stroke patients arrive at the hospital within the therapeutic time window, only 40% of these patients receive thrombolytic therapy with rtPA [5, 6]. This is due to the inability to accurately predict who will benefit from the treatment without suffering severe side effects, like hemorrhagic transformation (HT), which causes significant morbidity and mortality in stroke patients. The identification of those individuals that are likely to benefit from the therapy is dependent on a rapid and accurate diagnosis, which has not been developed yet. A set of protein biomarkers present in the blood at certain concentrations have revealed the potential to predict the right candidates for rtPA treatment [2, 7]. Cellular fibronectin (c-Fn) and matrix metalloproteinase 9 (MMP9) are biomarkers associated with the acute cases and relevant for patient stratification [5–9]. Besides, recently, we performed a clinical study using a magnetoresistive (MR) biochip platform that has proven to detect within the clinical and relevant dynamic range of those biomarkers [10]; the sample preparation is still a crucial step and very time-consuming. After separating serum from blood, the magnetic labeling of the target is performed inside a 1.5-mL Eppendorf™ tube that requires an incubation time of up to 1 h, therefore limiting the delivery of fast results.

Microfluidic chips are potential devices for sample preparation due to the low volume sample handling, biocompatibility, low-cost material and fabrication, disposable, and simple operation. Several studies reported the biochemical sample handling using microfluidic and magnetic beads to capture protein markers [9, 10]. In addition, one of the notable reports proposed a magnetic susceptibility-based protein detection using magnetic levitation [11]. However, large equipment, such as an optical microscope, is still required to observe the particle bindings. Therefore, this approach will be challenging to use on stroke patient stratification, which requires a fully portable device and preferable equipment-free, such as to avoid the utilization of a pump or bulky detection system.

Several studies reported various principles of driven flow of microfluidic devices to reduce the dependency of the bulky pump, such as surface tension method [12], osmotic pressure [13], and mini-pump lid. Nevertheless, several limitations of these technologies are not suitable to the biochemical assay used for stroke patient samples. First, the clinical sample waste will be preferable to be handled as simple as possible. Second, the passive pump will be favorable for the battery- and power-free purpose. Third, the required assay should be compatible with the practical use of conventional assay methods in hospitals. For example, the osmotic solution such as NaCl buffer is not preferable [13].

Another popular method to avoid the requirement of an electric pump in the microfluidic system is the capillary-driven chip [11, 14–16]. The capillary attraction can be driven by porous or cellulose materials, such as thread, woven fabrics, paper [17–22], and cellulose sponges [16, 23, 24]. Besides the low-cost and environmentally friendly materials, the cellulose-based capillarity also has a practical feature to handle liquid waste after the experiments.

To improve the magnetic target labeling and decrease the laborious work of the operator during the experiment, we developed a simple, miniaturized, and pump-free microfluidic chip for sample preparation to precede the MR detection system. The flow was driven by two-step micro capillarity, by hydrophilic serpentine channel and a sponge for sample waste absorbent. We demonstrate that it is possible to magnetically label the interested target in 15 min not compromising the number of protein biomarkers detected by the MR biochip platform. The simplicity of the microfluidic chip shows the potential of using it in conventional laboratories at the hospital for a first biomarker screening.

Materials and method

A simple microfluidic chip was developed to enhance the efficiency of magnetic labeling to the c-Fn and MMP9. The illustration of the proposed sample preparation chip is depicted in Fig. 1. The microfluidic was designed to be simple, pump-free, electric power–free, requiring low-cost material and fabrication, miniaturized size, and disposable.

Microfluidic chip fabrication

The mold of the sample preparation chip was designed using the two-dimensional (2D) vector software (ArtCAM® v.2011, Autodesk Inc.) aiming to have a total size of 75 × 25 × 5 mm and containing an inlet and outlet with a diameter of 7 mm and a height of 2 mm. The serpentine channel width and thickness are 500 μm and 150 μm, respectively; the total length of the channel (including the bending regions) is around 100 mm. The pre-concentrated chamber was designed with 10 mm of diameter and 150 μm of thickness. The final design of the mold was optimized and simulated for the engraving by end mill (Atis, Spain) cutter with diameter size of 0.8 and 1.5 mm, to compromise the parameters, such as the engraving speed, sequence, resolution, tolerance, and smoothness of rotation speed of the end mills.

Subsequently, the design was exported to the computer numerical control (CNC) high-speed milling machine (Flexicam Viper 606, FlexiCAM GmbH, Germany). The transparent polymethyl methacrylate (PMMA) plastic (Acrilico Cristal 10,400, Plexicril, Portugal) with a thickness of 5 mm was prepared to be engraved as the molding. Next,
the fabricated mold was cleaned by a wire brush followed by rising with isopropyl alcohol (Sigma-Aldrich, Merck KGaA, Germany) and N\textsubscript{2} gas blowing. The final mold was then inspected under the microscope (Wide-Field Upright Fluorescence Microscope—Nikon Ni-E, Japan) to examine the quality of the structure (Figure S1).

A PMMA plastic for the magnetic disc holder with a diameter of 15 mm and a thickness of 2 mm was also fabricated using a milling machine. Later, a disc-shaped magnet (supermagnet, Webcraft GmbH, Germany) was attached inside the cylindrical hole of the PMMA.

Polydimethylsiloxane (PDMS) elastomer chemicals (DowSill\textsuperscript{TM} 184 Silicone Elastomer, Dow Chemical, USA) with a ratio of 10:1 were mixed (3 min, room temperature/RT) and followed by degassing in the vacuum to remove the air bubbles. Next, the PDMS was cast to the mold to cover all the structures up to the same level of inlet and outlet thickness, before the curing process in the oven (2 h, 65 °C). The hardened PDMS was cut and peeled off from the mold. The channel-patterned PDMS and a cleaned soda glass substrate with a size of 75×25×1 mm (Epredia\textsuperscript{TM} Microscope Slides, Thermo Scientific, USA) were exposed by plasma O\textsubscript{2} (Expanded Plasma Cleaner PDC-002, Harrick Plasma, USA) for 40 s. The patterned PDMS immediately bond to the glass substrate with an assisted uniform pressure to all the PDMS surfaces.

Hydrophilicity treatment of the chips

The hydrophilicity treatment of the microfluidic channel was tested by using three different methods, i.e., channel passivated chip using: (1) 10% polyvinyl alcohol (PVA), herein this chip is termed as PVA-chip; (2) poly(dimethylsiloxane-b-ethylene oxide) methyl terminated (PDMS-b-PEO), mentioned as PEO-chip; and (3) the last method is by the additional 1% of PDMS-b-PEO while preparing the PDMS mixing, herein mentioned as PEO-m-chip.

The PVA (Sigma-Aldrich 363,073, Merck KGaA, Germany) was prepared in deionized (DI) water of 10% dilution; then, it was magnetically stirred for 40 min at RT. Next, the temperature was gradually increased to 100 °C for another 40 min on the stirrer, followed by 8 h of stirring at 65 °C. The PDMS-b-PEO (PDMS-b-PEO 09,780–100, Polysciences Europe GmbH, Germany) was diluted in the DI water with a concentration of 1% following by stirring the solution at RT for 15 min. The prepared PVA (or PDMS-b-PEO) solution was dropped in the inlet of the different fabricated chips, around 100 μL, respectively. Next, the chip was stored in the hot plate for 30 min at 40 °C, then 100 °C for PVA. For PDMS-b-PEO, the chip was stored at 65 °C in the oven until dry; then, this process was repeated three times. After the third drop of chemical passivation, the chip was stored in the oven (8 h; 65 °C). Finally, the PVA-chip
and PEO-chip were ready for the labeling process or can be stored at room temperature storage.

The third chip (PEO-m-chip) was prepared with a different recipe of PDMS mixing compared to previous chips. First, when the PDMS elastomer was mixed with a ratio of 10:1, additional drops of PDMS-b-PEO were added with a ratio of 1% from the total mixing volume. Next, the consecutive process was identical to the untreated chips, such as degassing, casting, curing, cutting, peeling, and bonding to glass by plasma O₂. Then, PEO-m-chip was obtained and ready for the labeling process. For the sample’s capillary attraction to the chip’s outlet, the ultra-fine PVA-sponge sheet (RamerFoam UD grade, Ramer Ltd., UK) with the density of the pores around 30 PPC (pores per centimeter) were cut into cylindrical shapes with a diameter of 10 mm. The cylindrical sponge is aligned to the outlet after the sample fulfills the pre-concentrated chamber.

Magnetic nanoparticle functionalization

The magnetic labeling functionalization was described in detail previously [8]. Briefly, streptavidin-conjugated 250-nm magnetic nanoparticles (MNPs) (2 μL of 4.9×10¹¹ mL⁻¹, Nanomag, Micromod, Germany) were incubated for 1 h with 50 μg/mL of 60 μL biotinylated polyclonal antibodies (Immunostep, Spain) of c-Fn or MMP9 (prepared in phosphate buffer with 0.05% Tween®20, PB-T). Next, the MNPs were blocked for 1 h with bovine serum albumin (BSA) 5% in PB. Subsequently, the MNPs were functionalized (Figure S2) as described previously [25–28].

Detection of MNP-labeled biomarkers on the magnetoresistive biochip

The MNPs of 1:1 or 1:4 were dropped together with the protein biomarker, c-Fn or MMP9, in the inlet of the microfluidic chip in a total volume of 100 μL (Fig. 1A).

ΔVbinding is the difference between the end signal (after incubation and washed step) and the initial baseline.

Statistical analysis

The data presented for each concentration is related to the average of the signal obtained from at least 20 different sensors, measured simultaneously in real-time, from a maximum of 30 sensors available at the MR biochip. The propagation of errors from active sensings was utilized to calculate the average signal value and its standard deviation (SD). Hillslope model was applied for the curve fitting of the calibration curve with 95% of confidence level, 1000 data points, ANOVA, and up to 400 iterations. The confidence signal level of LOD (Y_LOD) was calculated by [29]:

\[ Y_{LOD} = 1.645 \text{SD}_0 + 1.645 \text{SD}_1 \]  (1)

SD₀ and SD₁ are SD of blank sample and smallest concentration, respectively; subsequently, the Y_LOD value was substituted to the fitting curve to estimate the LOD concentration in x-axis of the curve.

Results and discussion

Hydrophilicity and microfluidic chip performance

After bonding to soda-lime glass, the fabricated microfluidic chips were tested for sponge capillary performance. When the first sample was loaded in the inlet of the original chip (without chemical treatment), the liquid automatically flowed to the serpentine channel due to the intrinsic hydrophilic properties of the serpentine channel created by the plasma O₂ treatment before the PDMS bonding to the glass. The plasma O₂ induced radical species of silanol groups (Si–OH), alcoholic hydroxyls (C–OH), and carboxylic acids (COOH) [30, 31]. This rich –OH surface is critical for covalently bonding to the soda-lime glass substrate, which also contains substantially –OH groups due to its composition formed by amorphous SiO₂ up to 70% [32]. Figure S3 and video V1 in the supplementary materials depict the assessment of hydrophilicity and capillarity of the fresh-fabricated device without any hydrophilicity treatment.

The abundance of –OH atoms on the surface of the channel and walls [33] maintains the hydrophilic properties which attract the capillarity of the sample delivery to fulfill the pre-concentration chamber. Once the sample complies with the pre-concentrated chamber and is attracted by the sponge, the absorption into the sponge occurs in less than 3 min. The flow rate was noted around 30 to 50 μL/min. The hydrophilic properties of the channel remained for up to 2 days, which is not useful for the long-term preservation of the device. This short lifetime of the hydrophilic surface...
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can be explained by the gradual release of -OH groups from the Si atom of the PDMS surface [34]. Thus, to improve the hydrophilicity lifetime of the channel, the PDMS was passivated using PVA [30] (for PVA-chip), or PDMS-b-PEO [35–37] (for PEO-chip), also using additional 1% PDMS-b-PEO materials during the material mixing of the PDMS, before the casting to the mold (for PEO-m-chip) (Table 1).

The PVA passivation treatment of the PVA-chip can be explained by the nature of covalent bonding between –OH groups (in the PDMS channel) and –C atoms (in the PVA). Moreover, the device baking (40–100 °C) after the PVA passivation enhances the oxygen-scavenging process deeper inside the PDMS material, and the early bonding of –OH forms a stable SiO2 bond (Scheme S1(A)). On the other side, the –COOH groups remain on the surface and are responsible for the hydrophilic characteristics [38]. The characteristic of the PVA-chip achieves a flow rate of 4.8 μL/min and hydrophilicity lifetime of up to 2 months.

For the PDMS-b-PEO hydrophilicity treatment of the PEO-chip, the mechanism can be described by the Van der Waals force and hydrophobic interaction between both PDMS blocks (PDMS block of the channel and PDMS block of the channel and PDMS block of the channel and PDMS block of the channel).

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**Table 1** Hydrophilicity treatments applied to the microfluidic chip to enhance the capillary sample flow and lifetime

| Hydrophilic treatment | As fabricated | PVA-chip | PEO-m-chip | PEO-chip |
|-----------------------|---------------|----------|------------|----------|
| Hydrophilic treatment | Plasma O2 during bonding | Channel passivation | Mix with the whole PDMS | Channel passivation |
| Temperature           | RT            | 40–100 °C | RT 65 °C   | 40–100 °C |
| Activation time        | 2 min         | 8 h      | No additional time | 8 h |
| Channel flow rate      | 30–50 μL/min  | 4.8 μL/min | 27 μL/min  | 25 μL/min |
| Advantage              | Simple, high flow rate | Strong bonding | Simple process | Medium flow rate |
| Drawback               | Short lifetime | Very low flowrate | Weak bonding, easy leakage | Activation time |
| Hydrophilicity lifetime| 1–2 days      | 2 months  | 2 months   | 2 months  |

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**Fig. 2**  
A Schematic of the magnetic labeling on-chip. Sample loading (1 + 2) of (1) functionalized MNPs with the (2) protein biomarker, and after the labeling on-chip, the end sample (3) MNPs with the interest biomarker captured.  
B The picture of the proposed microfluidic chip during the labeling process of protein biomarkers
block of the PDMS-b-PEO). Furthermore, the baking of the chip (65 °C) induces the permanent binding of both PDMS blocks, while the PEO blocks remain on the surface to maintain the hydrophilicity of the channel (Scheme S1(B)) [39].

In addition, for the PEO-m-chip treatment, the mechanism can be slightly different. PDMS-PDMS binding blocks are started during mixing of the elastomer, curing, and the PDMS-b-PEO materials. Next, the baking process enhanced permanent cross-linked PDMS blocks, while the PEO side remains hydrophilic on the surface [36, 39]. Thus, the surface of the whole PDMS material is hydrophilic in a stable state. Consequently, this modified PDMS is hard to be controlled by the plasma O₂ during the bonding process. Therefore, PEO-m-chip has a weak bonding substrate and makes the potential leakage in the channel. The hydrophilicity lifetime of the proposed chip could be improved up to 2 months, with its different flow rates as presented in Table 1. The comparative performance of the microfluidic chips shown in Table 1 highlighted the importance of finding a compromise between the different variables, i.e., the flow rate of the channel capillarity, the device lifetime, and bonding quality of the chip to the glass substrate. The indicated flow rates were measured until the sample fulfills the pre-concentrated chamber. After this step, the flow rates of the chips have relied on the capillarity grade of the sponge. The channel flow rate plays a vital role in giving the MNP and the protein biomarkers enough time to bind each other. Therefore, the fast flow rate will not be preferable due to the lower probability of binding in incubation time. In consideration of medium flow rate (25 μL/min), hydrophilicity lifetime up to 2 months, and non-leakage channel of the chip, the PEO-chip showed the required properties to perform the magnetic labeling assay in our study.

The labeling on-chip illustrated in Fig. 2 was performed in less than 15 min, starting from the sample loading in the inlet until the MNP extraction in the outlet, where MNPs are ready to use on the MR biochip platform. The efficiency of the magnetic labeling on-chip was compared with the conventional magnetic labeling (performed by the operator and using a 1.5-mL Eppendorf™ tube) by analyzing the detection of MNP-labeled targets on the MR biochip. For that, 30 spin valve sensors were functionalized, 15 sensors with the antibodies to capture the specific target c-Fn, and another 15 sensors used as control (with 5% BSA). The conventional and the labeling on-chip were tested using a high concentration of c-Fn (10 μg/mL). The results (Fig. 3A) showed that the labeling on-chip process (≤ 15 min) achieves a comparable signal level in the MR biosensor to the conventional labeling on-tube (1 h) from the previous reports [8, 40, 41].

While for the 15-min labeling on Eppendorf™, the signal level was significantly dropped around 50%. The efficiency of magnetic labeling on-chip

The high-efficiency labeling is a critical factor for our research in terms of time and the amount of captured targets. This purpose is relevant to the short time of the symptom onset of the stroke patients before the rtPA treatment.

Fig. 3 A Sensor signals representing the binding efficiency of magnetic labeling on-chip of c-Fn compared to the conventional labeling on 1.5-mL Eppendorf™ tube. The undiluted MNPs were exposed to 10 μg/mL of c-Fn. B The device characteristics by the different channel heights and its correlation to the operation time and labeling performance. The molding for channel characteristics were fabricated using 3D (three-dimensional) printing technology (Method S1 in Supplementary Materials).
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binding efficiency signals are demonstrated in Fig. 3A. Moreover, in Fig. 3B, we showed the correlation of channel height to the operation time and the binding efficiency from the MNP filtration in the pre-concentrated chamber. There is a trade-off between the operation time and labeling efficiency from the channel height characterization. The higher the channel height, the faster sample absorption into the sponge leads to the faster operation time of the labeling. Nevertheless, the labeling efficiency drops with the increase of the channel sizes. It can be explained that with the higher channel height, the larger surface area of the sponge interacts with the liquid and enhances absorption speed. On the other hand, a smaller number of targets can be filtered by the MNP cluster in the pre-concentrated chamber. In contrast, the shallower the channel height of the microfluidic device, the longer operation time is required for the labeling due to the lower absorption contact by the sponge. But it leads to better filtration by MNP cluster. Based on these findings, the proposed microfluidic device with a channel height of 150 um achieves the optimized labeling efficiency with a reasonable operation time.

To assess the protein binding on MR biochip, the density of MNP-labeled biomarkers can be observed visually under microscopic inspection. The several MNP clusters on the gold surface are represented by brownies (pointed out by arrows and other areas). In contrast, the control regions of the spin valve sensors (Fig. 4C and D) show clear areas from browny spots of MNP clusters. It shows that both labeling on-chip in 15 min proposed in this article can be comparable to the conventional labeling on Eppendorf™, by comparable numbers of brown spots of MNP binding in the sensor surface.

Fig. 4 Optical microscope representative images (Wide-Field Upright Fluorescence Microscope—Nikon Ni-E) of MNPs binding on the spin valve sensor surface (gold color), either using the microfluidic chip (A) or using the conventional magnetic labeling on 1.5-mL Eppendorf™ tube (B). The control regions of the spin valve sensor, which were blocked with 5% of BSA (C and D). The arrow shows the MNP clusters binds to the sensor surface. The illustration of the sandwich binding assay in MR sensing region for target (square blue) is depicted in (E)
Ischemic stroke biomarker detection in MR platform

The stroke biomarkers c-Fn and MMP9 were used as the case study to test the microfluidic chip combined with the MR platform. The clinical cutoff values of the c-Fn and MMP9 in stroke patients’ blood are 3.6 μg/mL and 140 ng/mL, respectively [42, 43]. Consequently, for the detection in the MR platform, a dynamic range of the sensor across the clinical cutoff value plays a critical role in foreseeing an actual clinical case scenario.

The MR biosensor was exposed to a series of concentrations of labeled c-Fn from 1 ng/mL to 10 μg/mL in PB buffer. Figure 5A (square blue curve) shows that using undiluted MNP (MNP 1:1) concentration, the detection limit is about 2.8 ng/mL and reaches the saturation value in the concentration of 1 μg/mL. This dynamic range does not fulfill the clinical requirement for c-Fn analysis. The saturation point is below the clinical cutoff value of 3.6 μg/mL, as indicated in the green dash line. The finding in our previous study [8] suggested that the concentration of the MNP and the magnetic focusing strategy can be tuned to adjust the dynamic range of the assay according to clinical needs. The 4 times dilutions of MNP (MNP 1:4 in Fig. 5A, square red curve) decreased the assay sensitivity by achieving the detection limit of 54.6 ng/mL and eliminating the saturation concentration at 1 μg/mL.

Compared to our previous report [8], the c-Fn detection in this assay started from a very small concentration (1 ng/mL) in PB buffer, which was not explored before until this range. Therefore, the potential performance and applications of the labeling strategy combined with the MR biochip platform can be observed, especially for other protein biomarkers with low clinical cutoff value requirements. Moreover, we found that for small concentration values, the labeling on-chip performs a slightly better MR signal compared to our previous study. This finding can be explained by (1) the use of PB buffer on the dilution of c-Fn that decreases the nonspecific binding effect compared to the use of depleted-serum; (2) the use of a miniaturized magnetic labeling chip that increases the probability to capture protein biomarkers due to the dynamic interactions in the serpentine channel, where biomarker-MNPs have more chance to interact; and (3) the filtration by MNP clusters (crescent-like formation) in the pre-concentrated chamber improves the probability to capture the target protein.

Furthermore, the MMP9 detection in an MR biochip platform using the proposed microfluidic chip for biomarker labeling is presented in Fig. 5B. The MMP9 biomarker labeling and detection are challenging due to its cutoff requirements and small molecular weight (95 kDa) [44] compared to c-Fn (440 kDa) [45]. This last aspect influences the obtained calibration curve of MMP9 using undiluted MNP and MNP 1:4. The dynamic range when using undiluted MNP shows, across the concentration of clinical cutoff value, a significant linear range with a LOD of 11.5 ng/mL. While when using MNP 1:4, the LOD was calculated around 132 ng/mL, which is very close to the concentration of clinical cutoff value (140 ng/mL). Therefore, for patient stratification using the MNP 1:4, the quantification of MMP9 present in healthy patients will be very difficult. Thus, for MMP9 detection, the use of undiluted MNP particles during the labeling process is preferable.

Overview and discussion of related technologies

To summarize the discussion and potential feature of the proposed microfluidic chip, we reviewed a previously published study of microfluidics utilizing magnetic field for labeling or particle separation to understand the novelty and potential applications of our device. A comparative table of these related magnetic microfluidics devices is listed in Table 2.
Zordan et al. proposed disposable microfluidics for multiplexed detection of *Escherichia coli* in SPR and fluorescence imaging [46]. In comparison, Lee et al. reported microfluidics for isothermal solid-phase amplification and detection of DNA methylation in 65 min of operation time [47]. Another method using 3D magnetic grid separation for *Salmonella* cell samples was reported by Hou et al., and it achieves an operation time around 1 h [48]. Tsai et al. proposed a magnetic field immunosensor by using a magnetic nanoparticle conjugated target immobilized on the microfluidic chip for IgG and carcinoembryonic antigen (CEA) samples. The sensing principle is quite simple and can be operated in less than 10 min. The separation of paramagnetic and non-magnetic cells is demonstrated for capturing live cardiomyocytes in a microfluidic device. The combination of the magnetic field, gravity, and hydrodynamic force from buffer flow inside the channel separates the paramagnetic cells and the targeted cells at around 20 min [51].

Another study presented a micromagnetic cell sorter for rapid capture target CD3+ T lymphocytes only around 20 s. Two permanent magnets are located on the opposite sides of the channel to deviate the flow of the biomarkers labeled by...
micromagnetic particles [52]. Furthermore, the Baeumner group presented magnetic labeling of nanovesicles on microfluidics devices in 15 min of operation time [54]. Another rapid capture of circulating tumor cells (CTC) of COLO205 and SKBR3 was reported in microchip-based immunomagnetic in 15 min [55].

Microfluidic cell separation from blood was demonstrated for the targeted white blood cell (WBC) for 4-min operation [56]. Subsequently, the non-lithography microfluidics was utilized for a multiplexed cancer biomarker screening of IL-6 and IL-8 around 30 min [57]. In addition, a portable, fully integrated, and miniaturized cytometer chip was used for the stratification of WBC and red blood cell (RBC) in 5 min [58]. Vojtíšek et al. reported the DNA hybridization on a microfluidics chip for 1-min operation time [59]. Nevertheless, the mentioned reports above are still dependent on using the pump, and the typical disposable part is the microfluidic device itself.

Interestingly, the proof-of-concept of the whole disposable sample preparation chip was demonstrated by Liang et al. [61]. This study reported single magnet manipulation for 3D focusing ferromicrofluidics. In addition, microfluidic devices were reported utilized for magnetophoresis for particle and cell separation. In this study, the Saccharomyces cerevisiae was used as a target biomolecule [60]. These studies are good examples of the whole disposable chip for sample preparation. Nevertheless, these proof-of-concept require magnetophoresis assay, where the captured target relies on the magnetic nanoparticle label followed by measurement to quantify the result.

The comparison of using proposed microfluidics for sample preparation in our study is strongly correlated to the screening of ischemic stroke biomarkers, especially for the potential application using clinical samples. Based on this purpose, this article emphasizes a microfluidic device with no additional equipment required, i.e., a pump and power source for simple operation and disposable format. It provides target labeling in less than 15 min and is strongly correlated with a portable MR biochip platform, in which sensors are sensitive to small magnetic fields generated by the MNPs. On the other hand, these MNPs allow for the capture and concentration of the interesting target from the complex sample matrix (serum), obtained, in our device, by manipulation of magnetic field using a permanent magnet.

**Conclusion**

In summary, we developed a microfluidics chip to perform high efficiency of magnetic labeling of ischemic stroke biomarkers, c-Fn, and MMP9, for the following detection by an MR biochip platform. By using the proposed magnetic labeling on the pump-free microfluidics chip, the incubation time can be reduced from 1 h down to 15 min. The efficiency of the labeling time is very critical for the screening of biomarkers of acute ischemic stroke patients because of the short therapeutic time window (within the 4.5 h). Moreover, the proposed labeling on-chip process is equipment-free (pump and power supply-free), provided in a miniaturized size, with a simple operation and sample handling, disposable, and low cost. Therefore, the proposed device has the potential to be integrated with a detection system based on MNPs and used as a point-of-care platform for the fast screening of biomarkers.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00216-022-03915-w.

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**Declarations**

**Conflict of interest** The authors declare no competing interests.

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