Self-driven efficient blood plasma separator microfluidic chip for point of care testing

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Article Keywords:	Lab on a Chip, Microchannel Integrated Micro-Pillars (MIMP), Point of Care Testing (POCT)
Abstract:	<p>Recently, there is a growing need for lab-on-a-chip devices in clinical analysis and diagnostics especially near the patient care. First step, in the most blood assays is plasma extraction from whole blood. This paper presents a novel high throughput blood plasma separation microfluidic chip, which with just a single droplet of undiluted human blood ($\sim 5\mu\text{L}$) can separate (more than $0.1\mu\text{L}$) plasma from whole blood without the need of external forces with high purity (more than 98%) and reasonable time (3 to 5 minutes). This would be the first step towards the realization of single use, self- blood test which does not require any external forces or connection to deliver and analyze a fresh whole blood sample in contrast to the conventional blood analysis which have variable waiting times. Polydimethylsiloxane (PDMS) is utilized as the base material to manufacture the microchip due to its biocompatibility and outstanding characteristics. PDMS has been modified with a strong nonionic surfactant (Silwet L-77) to achieve a hydrophilic behavior. The main advantage of this microfluidic chip design is the clogging delay on the filtration area, which results in an increased amount of extracted plasma ($0.1\mu\text{L}$). Moreover, the plasma can be collected in one or more $10\text{-}\mu\text{m}$-depth channels to facilitate the detection and readout of multiple blood assays. This high volume of extracted plasma is achieved; thanks to a novel design that combines the maximum pumping efficiency without disturbing the red blood cells (RBCs) trajectory by the use of different hydrodynamic principles such as constriction effect and symmetrical filtration mode. To demonstrate the microfluidic chip functionality, a novel hybrid microdevice, exhibiting the benefits of both microfluidics and lateral flow Immuno-chromatographic tests, is designed and fabricated. The performance of the presented hybrid microdevice is validated utilizing rapid detection of the thyroid stimulating hormone (TSH) within a single droplet of whole blood.</p>

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Self-driven efficient blood plasma separator microfluidic chip for point of care testing

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ABSTRACT

Recently, there is a growing need for lab-on-a-chip devices in clinical analysis and diagnostics especially near the patient care. First step, in the most blood assays is plasma extraction from whole blood. This paper presents a novel high throughput blood plasma separation microfluidic chip, which with just a single droplet of undiluted human blood ($\sim 5\mu\text{L}$) can separate (more than $0.1\mu\text{L}$) plasma from whole blood without the need of external forces with high purity (more than 98%) and reasonable time (3 to 5 minutes). This would be the first step towards the realization of single use, self- blood test which does not require any external forces or connection to deliver and analyze a fresh whole blood sample in contrast to the conventional blood analysis which have variable waiting times. Polydimethylsiloxane (PDMS) is utilized as the base material to manufacture the microchip due to its biocompatibility and outstanding characteristics. PDMS has been modified with a strong nonionic surfactant (Silwet L-77) to achieve a hydrophilic behavior. The main advantage of this microfluidic chip design is the clogging delay on the filtration area, which results in an increased amount of extracted plasma ($0.1\mu\text{L}$). Moreover, the plasma can be collected in one or more $10\text{-}\mu\text{m}$ -depth channels to facilitate the detection and readout of multiple blood assays. This high volume of extracted plasma is achieved; thanks to a novel design that combines the maximum pumping efficiency without disturbing the red blood cells (RBCs) trajectory by the use of different hydrodynamic principles such as constriction effect and symmetrical filtration mode. To demonstrate the microfluidic chip functionality, a novel hybrid microdevice, exhibiting the benefits of both microfluidics and lateral flow Immuno-chromatographic tests, is designed and fabricated. The performance of the presented hybrid microdevice is validated utilizing rapid detection of the thyroid stimulating hormone (TSH) within a single droplet of whole blood.

Keywords: Lab on a Chip, Microchannel Integrated Micro-Pillars (MIMP), Point of Care Testing (POCT)

1. Introduction

Lab on a chip devices have become very attractive and also are growing in clinical analysis and diagnostics due to their outstanding virtues (rapid operation, low requirements for samples and reagents, high convenience and low cost) [1].

Many pathologies and physiological conditions can be diagnosed through the analysis of blood plasma which provides crucial information of various internal organs [2, 3]. For instance, various proteins have been found as biomarkers for the diagnosis of multiple cancers [4] or circulating nucleic acids (CNAs) have been shown an excellent benefit for the diagnosis of

malaria [5], stroke [6] and several cancers [7]. To reduce the time from blood collection to the test and provide faster and yet less expensive and comprehensive results, “lab-on- a-chip” type of devices are an attractive tool for blood plasma separation and analysis, compared to traditional centrifugation processes.

Currently, microfluidic blood plasma separation techniques can be classified in three main formats: paper-based microfluidics, the CD and the microfluidic chip format [7]. Paper-based microfluidic were widely used in lateral-flow assays, and recently the use of paper to build microfluidic circuits shows a great potential to

create low-cost miniaturized blood analysis devices [9, 10, 11]. But, this technology is relatively new, which means issues such as the control over flow rates, mixing and interaction times between sample and reagents have not yet been perfected.

The CD based format has the potential to become a standard tool for blood plasma separation and micro total analysis systems (μ TAS) [12, 13] but with their own limitations, such as: the need of numerous valves, the need of a high-speed spinner and electrical power to manipulate the fluid.

Besides these two approaches, there are different microfluidic technologies summarized in the following reviews [14, 15, 16]. The microfluidics proposed designs to achieve plasma separation in chip format, which can be divided in two main categories: passive and active methods.

Active methods need an external force field such as acoustic [17, 18], electrical [19, 20] and magnetic field [21, 22] to achieve separation. In the category of passive separation methods, researchers exploit several techniques such as sedimentation [23, 24], dead-end filtration [25], cross-flow filtration [26, 27], deterministic lateral displacement [28, 29], pinched flow fractionation [30] and biomimetic separation methods [31, 32].

Since 2005, the attempts are focused to generate microfluidic designs that are self-driven by increasing the fabrication accuracy to provide more plasma output. For instance, Crowley et al. reported a capillary driven in-plane cross-flow plasma separation design from whole blood [33]. In 2009, Gervais et al. highlighted the usefulness of a capillary-driven multiparametric microfluidic chip for one-step immunoassays [34]. An embedded pad utilized to filtrate the blood plasma, which needed to load 20 microliters of blood sample because of the retaining some parts of the initial blood sample. In 2010, Chang Kim and his colleagues [35] presented a surfactant-added PDMS design, (PDMS+Silwet L-77), which used an out-of-plane cross-flow filter to extract plasma from whole blood. But, the volume of the extracted plasma was very small in the order of 20nL. In 2011, Dimove et al.

demonstrated a stand-alone microfluidic chip that extracted plasma from a 5 μ L droplet of blood and integrated an ELISA assay to detect biotin levels in 10 minutes [36] using sedimentation in a trench structure. Later, in 2012, Sakamoto and their colleagues proposed a capillary driven design working on the wetting properties of PMMA [37]. 3 % in volume of plasma from a 5 μ L of blood was achieved, but the instability of the PMMA surface modification process and RBCs clogging in the entrance of microchannel array are challenges, which should be overcome to use this approach in point-of-care (POC) manufacturing.

More recently, in 2013 a pump-free and membrane-based blood plasma separator is reported by Liu et al. [38]. Gravitational sedimentation and filtration techniques were combined for nucleic acid amplification and HIV detection. However, a large sample (1.8mL) is needed and further manipulation of plasma once separated is required. The same year, 2013, Kang and colleagues [39] developed a cyclic olefin copolymer (COC) whole blood plasma separator driven by asymmetric capillary forces. This chip separates approximately 102 nL from a single drop of 3 μ L (3.4%) whole blood within 10 min, but required a complicated post processing to achieve the filtration (series of sprayed hydrophilic barriers on the hydrophobic substrate).

Despite all these attempts, it is clear that there is still a lack for a simple device that addresses all the necessities for building a batch manufacturable POC device such as: separate plasma from untreated fresh blood, with a long shelf life and a reasonable separation time.

In this work, we design, fabricate and validate through an immunological test for the qualitative detection of the TSH (thyroid stimulating hormone) a novel blood plasma separation microfluidic chip. Hydrodynamic behavior of red blood cells in a hydrophilic PDMS constriction channel and plasma extraction using a symmetric out-of-plane cross-flow filtration MIMP (Microchannel Integrated MicroPillars) channel are used to maximize the amount of extracted

Figure 1. Schematic diagram of the blood plasma separation microdevice. The yellow arrow shows capillary forces on the extracted plasma in the MIMP filtration channel while the red arrow shows the shear force on the RBCs due to the constriction design. The main channel is included transport channel ($300\mu\text{m} \times 10\mu\text{m}$) and constriction ($100\mu\text{m} \times 10\mu\text{m}$) while two plasma collected channels ($100\mu\text{m} \times 10\mu\text{m}$) are embedded on both sides of the main channel (See video S-1 in the SI)

plasma from untreated blood. The presented microdevice has several advantages over the mentioned devices for blood plasma separation. This microdevice is self-driven which means that the capillary forces are sufficient to deliver and separate more than $0.1\mu\text{L}$ of blood plasma from a $5\mu\text{L}$ droplet of real fresh blood (required sample for blood testing in a POC device in terms of quantity and quality). Moreover, it does not require filters, tubes, plasma manipulation and due to its simple design it can be easily mass produced.

This paper is organized as follows: design and principles of the self-driven microfluidic device along with the fabrication procedure is described in Materials and methods section; the proposed blood plasma separation microfluidic device is experimentally tested and validated in Results and discussion section which also included the demonstration of the separated blood plasma quality with analyte detection.

2. Materials and methods

2.1. Design and principle of the self-driven microfluidic device

The proposed design combines several aspects such as separation science, fluid dynamics and blood rheology. The red blood cell clogging is an inevitable and major issue in blood passive filtration, which can be modulated by increasing the shear rate on the trapped cells that can sweep them away from the entrance of the filtration channel.

Figure 1 shows a schematic view of the microfluidic device design, which is manufactured in two parts. The top part comprises a main channel (called transport channel for delivering the blood drop sample with height, $10\mu\text{m}$) and two plasma collected channel with the same height. The microdevice down part (called MIMP filtration channel) includes an arrangement of diamond microposts to decrease the flow resistance as well as to increase the capillary force. The proposed MIMP filtration channel (down part design) not only filtrates RBCs but

also paves the path for collecting the separated plasma in the top part using multiple collected channels due to a filled distributed area with blood plasma. Its design is the result of our previous work for developing a high-throughput microcapillary pump with efficient integrated low aspect ratio micropillars [40]. The obtained results indicated that the diamond pillar array presents the minimum resistance flow compared to the other pillar shapes. Furthermore, because of the deformability of human red blood cells, the down part depth of the device, H_{FC} , is designed to be less than $2 \mu\text{m}$ to successfully filter the red blood cells ($1.5 \mu\text{m}$).

Two different transport channel designs have been investigated and compared, straight and curved design, see Figure 2a. In the transport channel straight design, the blood flow in the transport channel is perpendicular to the extracted blood plasma flow while in the curved design both flows are parallel.

The fluidic behavior of the whole device can be analyzed using an equivalent electrical network (Figure 2b). Where R_{T1} and ΔP_{T1} are the flow resistance and pressure drop of the first part of the transported channel (before constriction channel), R_C and ΔP_C are the flow resistance and pressure drop of the constriction channel, R_{T2} and ΔP_{T2} are the flow resistance and pressure drop of the second part of the transported channel (After constriction channel), R_F and ΔP_F are the flow resistance and pressure drop of the MIMP filtration channel and R_{PC} and ΔP_{PC} are the flow resistance and pressure drop of the plasma collected channel respectively.

Once the hydrophilicity of the material is fixed (surface tension), the capillary pressure ΔP for each of the rectangular microchannels can be calculated using the Young-Laplace equation:

$$\Delta P_i = \sigma \cos \theta \left(\frac{1}{h} + \frac{1}{w} \right) \quad (1)$$

where σ is the surface tension, θ is the contact angle, h is the half height and w is the half width of the microchannel respectively [41]. In the other hand, the flow rate Q of a liquid in a capillary

microchannel depends on the viscosity of the liquid, the capillary pressure and the microchannel flow resistance as following:

$$Q = \frac{1}{\mu} \frac{\Delta P}{R} \quad (2)$$

Figure 2. a) Proposed transport microchannel design layouts b) The equivalent electrical network of the microdevice c) Diamond shaped post array in the filtration area.

where ΔP is the pressure difference between inside and in front of the liquid (eq. 1), μ is the viscosity of the liquid and R is the microchannel flow resistance [41].

On this basis, the minimization of the MIMP channel flow resistance R_F will desirably increase the plasma flow rate in the MIMP filtration channel. To reach this aim, according to Madadi et al. [40]; the diamond post shape with increased side distance between pillars is the most efficient design for minimizing the MIMP channel flow resistance and it can be calculated through

$$R_F = \frac{12\mu L}{wH^3} \left(\frac{1}{2\epsilon - 1} \right) \quad (3)$$

Figure 3. a-c) MIMP filtration channel manufacturing steps. The microdevice top and down parts d) before and e) after plasma bonding

where μ is the fluid viscosity, L is the length of microchannel, w is the half depth of microchannel, H is the height of microchannel and ϵ is the porosity [40]. Since the fluid and the height of the channel are fixed, we have maximized the porosity to achieve a manufacturable device. Figure 2c shows the dimensions of the selected MIMP filtration channel.

2.2. Fabrication procedure

The microfluidic device is manufactured in two main parts: the top part and the bottom part, which are fabricated using different procedures.

2.2.1. Top part manufacturing process

SU-8, a negative tone photo-epoxy was employed to create a master mold using photolithography. Polydimethylsiloxane (PDMS), as a common material in microfluidic chips, was utilized for manufacturing the microdevice top part due to its outstanding characteristics (including its elastomeric properties, biocompatibility, gas permeability, optical transparency, ease of bonding to glass and low manufacturing cost)

[42, 43] by means of single soft lithography process. In spite of all the favorable physical and chemical properties, the hydrophobicity of PDMS is a handicap when pumping aqueous solutions through microchannels just by capillary forces. To overcome PDMS hydrophobicity and to control the PDMS hydrophobic recovery over a long period of time, nonionic surfactant (Silwet L-77) is added to PDMS before the curing process [44, 45]. The modification of PDMS hydrophobicity using surfactant not only guarantees the shelf life of the final POC device (because of the negligible hydrophobic recovery) for mass production but also eliminates the non-specific absorption of proteins or biological material. Further details on the fabrication process are explained in the supplementary information.

2.2.2. Down part manufacturing process

The MIMP filtration channel was fabricated on a glass substrate using both photolithography and wet chemical methods. Fig. 3 (a-c) illustrates the fabrication steps of the MIMP filtration channel. Further details on the fabrication process are explained in the supplementary information.

Figure 4. Photograph of *I*) PDMS part (top part), *II*) glass part (down part) and *III*) final microdevice

2.2.3. *Microdevice assembly and test strip integration.*

The PDMS microchannels (top Part) and the etched glass MIMP filtration channel (down part) were bonded via an oxygen plasma (Gambetti, Italy) treatment (See Fig.3d-e). Oxygen at 200 Torr was introduced in the oxygen plasma chamber (Gambetti) and the plasma was run for 30 seconds. The oxygen plasma parameters were chosen to achieve the maximum bonding strength [46]. Fig.4 shows photograph of the completed blood plasma separation microfluidic device.

To demonstrate and validate the quality of the separated plasma, the microfluidic chip has been combined with a lateral flow immuno-chromatographic strip for Thyroid Stimulating Hormone (TSH) test (NADAL® TSH Test, Germany). This test measures the levels of TSH, a hormone that is produced and released by your pituitary gland. The pituitary can sense whether there is enough thyroid hormone in the bloodstream, and it releases TSH when it detects insufficient thyroid hormone. The official normal

range for TSH in blood runs from approximately 0.5 to 4.5/5.0 µIU/mL. Therefore, a TSH under 0.5 µIU/mL indicates hyperthyroidism (an overactive thyroid), and a TSH over 5.0 µIU/mL indicates hypothyroidism (an underactive thyroid).

The separated plasma was driven to the entrance of half of a TSH test strip see figure 5. The filtration pad (fiberglass) and the absorption pad (in the end of test strip) have been removed from the embedded test strip. The test strip gold conjugate pad was overlapped with the blood plasma reservoir and sealed with tape.

In the gold conjugate pad the plasma antigens are recognized and bound to the gold-conjugated antibodies, see Fig. 5a. When the gold nanoparticle complex reach the test line, they form strong bond with the immobilized antibodies embedded in the nitrocellulose membrane and a colored line will appear.

Since the required volume of the blood plasma for wetting the conjugate pad is relatively high, 10µL of phosphate-buffered saline (PBS, purchased

Figure 5. a) Schematic view of the microfluidic chip including LFA strip b) The embedded steps of TSH test strip inside the microfluidic device

from Life Technologies S.A., Madrid, Spain) are added to the LFA strip after the plasma has reached the conjugate pad.

2.2.4. Sample preparation

Fresh human whole blood collected from a healthy volunteer using finger prick method was used for experiments. The sample was collected using a lancet of a POC device in every experiment. For the TSH blood test, two different types of blood sample were prepared. The first one; a 5- μ L drop of healthy fresh human whole blood and the second type; a 5- μ L drop of high concentrated TSH sample which was obtained from a reconstituted blood by mixing a high TSH dried plasma (provided by GRIFOLS) with RBCs from regular blood. Then, the collected blood samples were mixed with 0.5- μ L Heparin (purchased from Pfizer Co., Madrid, Spain) as a standard anticoagulant.

2.2.5. Experimental setup

In order to record images and movies of the blood plasma separation process and analyze the results, a digital camera (Tucsen ISH500, 5.0 M pixel,) was connected to a micro-inspection lens system Optem zoom 125C (with a broad 12.5:1 zoom range) and a 20X objective. All quantitative data were analyzed and expressed as the mean $\pm$ standard deviation.

3. Results and discussion

In order to study the effect of the transport channel geometry on the blood plasma separation, two different channel geometries were considered: A) Straight Transport Channel, B) Curved Transport Channel. The curved design helps to maximize the first instance filling rate of MIMP channel before the RBCs clogging of MIMP entrance due to the unidirectional of the blood flow and extracted plasma flow. The experiments were performed three times for each channel-geometry. Results are summarized in Table I,

Figure 6. Blood plasma separation process using the curved transport channel geometry a) The hollow transport channel (Blood Channel) and Plasma collected channel before introducing the whole fresh blood in the microchip inlet b) The filled blood channel by fresh blood and simultaneously filled MIMP filtration channel by separated blood plasma c) The starting point of blood plasma filling in the plasma collected channel d, e, f) The blood plasma filling process in the plasma collected channel g, h) The complete filled plasma collected channel by the extracted blood plasma between 5 to 6 minutes(See Video S-2 in the SI)

showing the average of the recorded data (see Supplementary information for the whole data).

3.1. Curved transport channel

A 5-μL drop of fresh human whole blood is introduced into the inlet port of the transport channel. The blood sample is drawn into the microdevice only by capillary forces due to the hydrophilicity of surfactant-added PDM (Silwet L-77). The separation process starts as soon as the blood flow in the transport channel arrives to the MIMP filtration channel. Since the MIMP filtration channel depth is less than 1.5 μm, RBCs do not penetrate into the plasma channel. In the separation zone, the blood flow velocity in the transport channel is slowed down which leads to an accumulation of cells in the entrance of the

separation area. The RBCs accumulation and clogging of the separation area is prevented due to a designed constriction channel in the middle of

Figure 7. a) A comparison between RBCs concentration in the main blood channel and the plasma collected channel b) The effect of the curved geometry and resulting centrifugal force on the RBCs (See Video S-2, SI).

transport channel, which generates local flow acceleration on the RBCs.

Using the curved transport channel design, even though the constriction effect tries to postpone the RBCs clogging; the centrifugal force due to the curvature and the nonsymmetrical arrangement of the separation zone leads to a precocious RBC clogging of the MIMP filtration channel entrance. Fig. 6 illustrates the filtration performance for the plasma separation from whole blood in the curved transport channel. By utilizing a curved transport channel, an average of 0.07 μL of blood plasma was extracted from 5 μL fresh whole blood.

In the separation process, the MIMP filtration channel was filled in the first minute, see Figure 6b (since the plasma flow direction is parallel to the blood plasma flow in transport channel) while the plasma collected channel was filled after 5 to 7 minutes, see Figures 6g and h. Figures 6b and 6c show that the plasma collected channel was filled firstly by the top surface of the pillars in the collected channel due to the higher capillary force compared to the other zones. It is noticeable that although some air bubbles are formed inside the separation area and in the plasma collected channel during the first minutes, they will disappear in the following steps of the process because of the PDMS permeability. Obviously, these trapped air bubbles will reduce the separation efficiency by creating a resistance to the capillary flow but it should be considered that the air bubble forming can be controlled by unifying the wetting properties of the modified PDMS and the glass [47]. The purity of the extracted blood plasma was defined as:

$$\text{Purity} = 1 - \frac{C_P}{C_M} \quad (4)$$

where, C_M is the number of RBC in the main channel and C_P is the number of RBC in the plasma collected channel [8].

The purity of the extracted blood plasma from whole blood in the curved transport channel topology is around 80 to 85%, using image processing, see figure 7a. After filling the plasma collected channel, 90 images were taken from three different regions (including the first, middle and the end) of the plasma collected channel using the described experimental setup. The ImageJ software was utilized for counting the number of

red blood cells in the same specified area (the considered volume is $5 \times 10^5 \mu\text{m}^3$) of every image region. The average of the obtained results (number of red blood cells in the plasma collected channel) compared to the hematocrit of the initial sample (fresh whole blood), which was 45% in our experiments.

Although the curved transport channel fills the separation MIMP channel quickly (less than 1 minute), the centrifugal force on the RBCs attributed to this geometry along with the nonsymmetrical capillary forces generate accumulation of RBCs in the entrance of MIMP filtration channel and secondly drive them into the MIMP channel and thereby in the plasma collected channel (See Fig 7b). A video clip of the performance of the blood plasma separation microdevice using curved channel is provided in Video S-2 in the supplementary material.

3.2. Straight transport channel

In order to increase the separation efficiency and purity of the extracted plasma and avoid the effect of the centrifugal force due to the curvature design, the straight transport channel was tested. An example of the microdevice performance can be seen in Figure 8. The mechanism of separation is similar to the curved transport channel. The only differences in this design are related to the direction of the blood flow, which is perpendicular to the extracted plasma channel flow, and the capillary forces which are symmetrically applied on both sides of the RBCs flowing in the transport channel. Therefore, the RBC trajectory is not greatly disturbed due to the symmetrical plasma extraction and consequently, the clogging of the filtration area is delayed owing to the elimination of curvature centrifugal force. In this case an average of 0.09 μL of blood plasma was successfully separated from 5 μL of whole fresh blood. According to the results, the plasma collected channel was filled in 3 to 5 minutes with purity of more than 98%. The results confirm that the perpendicular plasma flow decreases the required time to fill the collected channel [48]. A video clip of the performance of the blood plasma separation microdevice using straight channel is provided in Video S-3 in the supplementary

Figure 8. . Blood plasma separation process using the straight main channel geometry a) The hollow transport channel (Blood Channel) and Plasma collected channel before introducing the whole fresh blood in the microchip inlet b) The filled blood channel by fresh blood and simultaneously filled MIMP filtration channel by separated blood plasma c) The starting point of blood plasma filling in the plasma collected channel d, e, f) The blood plasma separation and filling process in the plasma collected channel g, h) The complete filled plasma collected channel by the extracted blood plasma after 3 minutes(See Video S-3 in the SI)

material. Table 1 shows a comparison of the obtained results between the straight and curved transport channel geometries.

To investigate the effect of passive capillary filling flow in the microdevice performance, three types of microdevice with different surfactant concentration (Silwet L-77 concentration 1%, 2% and 3% in PDMS) were fabricated by comparing the filling flow rates. More details about the experiments and the obtained results are explained in the supplementary information. According to the obtained results, the whole microdevice performance time was decreased more than 25% to less than three minutes through increasing surfactant (Silwet L-77) concentration from 1% to

3%. This minimization of the sample preparation time for blood plasma separation can be very effective to preserve the quality of analytes in the separated blood plasma as a bottleneck for blood analysis process in a POC device.

3.3. Double embedded plasma collected channel

For further investigation about the capability of the presented microdevice to perform simultaneously different assays on the same blood sample, another plasma collected channel was added at the other side of the main blood flow (1 mm apart). The filling of this second plasma collected channel not only opens the possibility to perform multifunctional blood analysis, but also

Table 1. Comparison between the straight and curved transport channel geometries

Microchip	Blood Sample Volume	Average volume of extracted Plasma	Average Time (minute)	Purity %
Straight Transport Channel	~5 μ L	0.09 μ L $\pm$ 0.01	4 $\pm$ 1	> 98
Curved Transport Channel	~5 μ L	0.07 μ L $\pm$ 0.01	6 $\pm$ 1	80-85

the volume of extracted plasma was increased up to 0.1 μ L from 5 μ L drop of whole fresh blood with the same purity (more than 98%). Since the presented blood plasma separation device is driven just by capillary forces, no hemolysis has been observed. More details about the hemolysis study can be found in the supplementary information. Moreover, a video clip of the microdevice performance of the double embedded plasma collected channel is provided in Video S-4 in the supplementary material.

3.4. Integration of plasma separation with analyte detection for TSH blood test

To demonstrate the utility of the quantity and quality of extracted plasma, the plasma from the collected channel is driven to the entrance of a lateral flow assay (LFA) to qualitatively detect Thyroid Stimulating Hormone (TSH) level. Two different type of test were performed based on the 5 μ L volume of the prepared samples; healthy fresh blood and reconstituted blood. Fig. 9 shows an example of the results of the TSH test using both the test strip device and the hybrid microfluidic chip (implemented test strip in the presented microfluidic device). It should be highlighted that in the Fig.9, the hybrid microfluidic chip is shown from the back side view due to the rotation of the test strip in the implementation process, see Fig. 5b.

Three test devices were tested for every sample type and the coloured line appeared in the control section of the test strip 18 $\pm$ 2 minutes after dropping the blood droplet. Figure 9a shows a picture of the obtained results from the hybrid microfluidic chip compared to the standard NADAL[®] TSH Test, which is shown in Figure 9c. As it can be seen from both devices the TSH test

result is negative and the appeared coloured line in the control 'C' section confirms the correct operation of the TSH test in the presented hybrid microfluidic chip. Figure 9b shows the results when a high TSH blood was processed, in this case the positive line plus the control line appeared.

Figure 9. The results of the TSH test using: a) Negative TSH result using a Hybrid microfluidic chip. b) Positive TSH result using a Hybrid microfluidic chip. (c) Negative TSH result using LFA strip.

According to the results, the proposed design shows its potential as a clinical tool compared to previous works. Firstly, the initial volume of the required sample, 5 μ L, is considerably reduced compared to the former works [34, 38]. Secondly, the volume of the extracted blood plasma, 0.1 μ L, is enough to implement the TSH test and also is more than the obtained volume in the previous filter designs [33, 35]. Importantly, the use of a simple design which is only made of two parts without post processing (such as previous works [37, 39]) or embedding additional part for filtration [34], makes it suitable for batch manufacturing. Furthermore, the capability of the presented microdevice for separating and gathering blood plasma within more than one

plasma collected channel pave the way for blood panels in a single POC.

4. Conclusion

In this work a novel high throughput blood plasma separation microfluidic chip was designed, built and validated through the integration of TSH qualitative test. The test could work with just a single drop of whole blood (~5µL) and 0.1 µL of blood plasma were successfully separated and collected in 10-µm-depth channels between 3 to 5 minutes with purity more than 98%. The long term hydrophilicity of the employed material, the straight forward manufacturing process and the achieved plasma volume (0.1 µL) pave the way for multiplex blood analysis simultaneously in the same device as the promised portable medical point-of-care-testing (POCT) for doing different blood analysis.

Supporting information

Additional information as noted in text.

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