Transactions of the Indian National Academy of Engineering (2020) 5:217–220
https://doi.org/10.1007/s41403-020-00123-9

TECHNICAL NOTE

Blood Plasma Microfluidic Device: Aiming for the Detection of COVID-19 Antibodies Using an On-Chip ELISA Platform

Siddhartha Tripathi[1] · **Amit Agrawal**[2]

Received: 13 May 2020 / Revised: 27 May 2020 / Accepted: 5 June 2020 / Published online: 10 June 2020

Abstract
COVID-19 is a public health emergency of international concern. Detection of SARS-CoV-2 virus is an important step towards containing the virus spread. Although viral detection using molecular diagnostic methods is quite common and efficient, these methods are prone to errors, laborious and time consuming. There is an urgent need for blood-based tests which are simple to use, accurate, less time consuming, portable and cost-effective. Human blood plasma contains water, proteins, organic and in-organic substances including bacteria and viruses. Blood plasma can be effectively used to detect COVID-19 antibodies. The immune system generates antibodies (IgM/IgG proteins) in response to the virus and identification of these antibodies is related to the presence of the infection in the patient in the past. Therefore, detecting and testing the presence of these antibodies will be extremely useful for monitoring and surveillance of the population (Petherick, Lancet 395:1101–1102, 2020). Herein, we describe and propose a microfluidic ELISA (enzyme-linked immunosorbent assay) system to detect COVID-19 antibodies on a lab-on-chip platform. We propose to first separate plasma from whole human blood using a microfluidic device and subsequently perform the detection of antibodies in the separated plasma using a semi-automated on-chip ELISA.

Keywords Microfluidic · ELISA · Antibody · IgM/IgG · SARS-CoV-2

What Is the Technology

The technology presented comprises a microfluidic blood plasma separation device which is capable of effectively separating plasma from whole human blood. Human blood constitutes cells and plasma. Blood plasma is considered an important source of information pertaining to human health condition; this is due to the presence of important bio-markers. Human blood plasma contains water, proteins, organic and in-organic substances including bacteria and viruses. Blood plasma is separated from other constituents on a routine basis. Use of plasma is preferred over whole blood in several diagnostic tests; this is due to clogging, cell lysis and cell interference issues associated with whole blood testing.

We have developed a passive microdevice to separate blood plasma; the device design and other features are shown in Fig. 1. The device is simple, compact and efficient. The principle behind plasma separation revolves around harnessing the bio-physical and geometrical effects of blood flow within a microchannel. Experimental results indicate that almost pure plasma (separation efficiency 99.5%, purity) is obtained by injecting whole blood at a flow rate of 0.5 ml/min. The yield (or amount of plasma obtained to amount of blood infused) of the device is 1% with whole human blood. The plasma separated was found to be hemolysis free and few biomarkers of interest, namely proteins, hCG (human chorionic gonadotropin) hormone, and glucose were successfully recovered from the separated plasma. The device was fabricated in PDMS using photolithography and soft lithography techniques (other fabrication materials and techniques can also be employed). The major advantage of such microdevice is its accuracy, ease of operation, use of small sample amount, small size, portability and ease of its integration with a bio-sensing platform. The device has been extensively studied, patented and has been reported in various publications (Tripathi et al. 2013, 2015a, 2015b, 2016,

✉ Siddhartha Tripathi
siddharthat@goa.bits-pilani.ac.in

[1] Birla Institute of Technology and Science, Pilani, K. K. Birla Goa Campus, Goa 403726, India

[2] Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

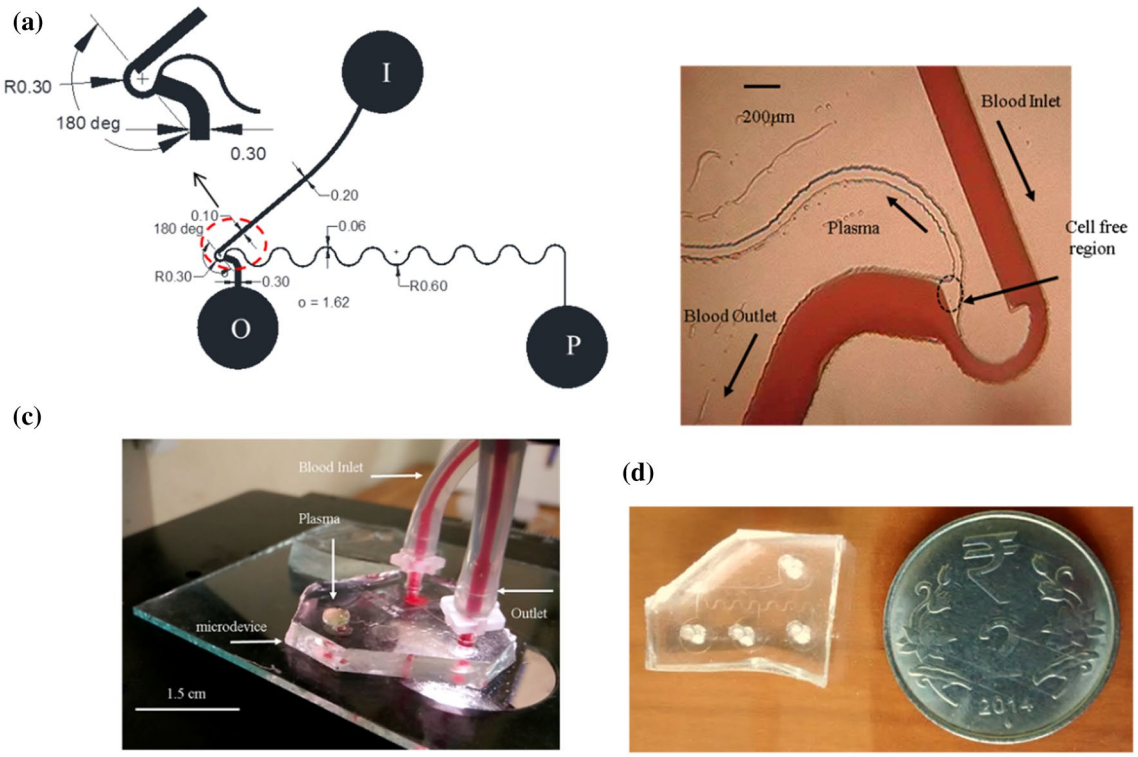

Fig. 1 **a** Blood plasma microdevice design and zoomed view at the junction. Symbols: I—blood inlet, O—blood outlet, P—plasma outlet. **b** Experimental photograph showing plasma separation in the microdevice using whole blood at a flow rate of 0.5 ml/min. **c** External view of the blood plasma separation taking place in the PDMS microdevice. **d** Comparison of the device size with a coin (Tripathi et al. 2016)

2018; Prabhakar et al. 2015). Recently, this microdevice has been successfully employed by a research group for measuring dopamine from whole blood. Researchers have successfully integrated this plasma separation microdevice with an enzyme-free plasmonic neurotransmitter dopamine biosensor to measure dopamine concentration with high detection selectivity (Vázquez-Guardado et al. 2018). The reported plasma separation microdevice is not only an alternate to the centrifuge, but it can also be easily integrated with a biosensing platform/detection technology (for example, ELISA) and result in a point-of-care device. Microdevice ensures separation of high-quality plasma with minimal cell interference enabling selection of an analyte with high specificity and sensitivity.

Novelty of Technology and for What It Was Made

The technology was developed to realize a microdevice to enable blood plasma separation in a lab-on-chip format in an effective way. The study was motivated from the current worldwide effort of developing point-of-care microdevices. There are numerous novel features of this microdevice. The developed microdevice is passive and does not rely on active techniques of separation, the device uses elevated dimensions, so maintaining tight tolerances is not essential; the design is, therefore, easy to fabricate and is cost-effective. The device can work efficiently over a wide range of hematocrit, both whole blood and diluted blood can be used. Whole blood is preferred as it ensures sufficiency of bio-markers in the separated plasma. The device can separate plasma in a continuous manner without clogging the microdevice. Approximately 10 μL of plasma can be removed using 1 mL of whole human blood in approximately 3 min. The device can easily be integrated with a bio-sensor to enable on-chip detection of a target analyte.

How the Technology Can Be Tweaked to Make It Relevant to COVID-19

The most common tests to detect the SARS-CoV-2 virus are the RT-PCR test (Swab based) and the antibody test (blood based). In addition, field effect transistor (FET)-based sensors for point-of-care testing have been reported. Recently, Seo et al. (2020) have reported successful detection of SARS-CoV-2 virus with high sensitivity in swab specimens

using a FET-based biosensor. The graphene-based device used SARS-CoV-2 spike antibody to detect the antigen protein. The swab-based tests directly detects the virus and are useful for early detection of the virus whereas blood-based tests are indirect and informs about the infection in the past. The technology of blood plasma separation in a microdevice can be employed for testing of antibodies present in the blood plasma of a COVID-19-infected subject, similar to a point-of-care serological test. The immune system generates antibodies (IgG/IgM proteins) in response to the virus. Identification of these antibodies is related to the presence of the infection in the patient in the past. Therefore, detecting/testing the presence of these antibodies will be extremely useful for surveillance of the population and also for plasma transfusion to combat the active virus (Petherick 2020). Also, blood-based test can allow for the measurement of additional bio-markers of interest such as CRP (C-reactive protein). This protein has been found to correlate with the severity of COVID-19 infection (Vashist 2020).

In the past, various researchers have reported detection of HIV, Zika, Hepatitis B, Dengue, Influenza, measles and rubella using microfluidic techniques (Yeh et al. 2014). Immunoassays can be used to measure small amounts of analytes effectively (Vashist 2020; Yeh et al. 2014; Lee and Lee 2013; Hsu et al. 2014; Liu et al. 2017). Herein, we propose the integration of sandwich ELISA (enzyme-linked immunoabsorbent assay) with the blood plasma separation microdevice to detect COVID-19 antibodies after minor modifications in the design. Although the separated plasma from the chip can be analyzed in a conventional ELISA, we prefer to propose the on-chip detection of analyte for simplicity and cost-effectiveness. The idea, procedure and steps have been presented in Fig. 2. The whole setup will be similar to an ELISA on a microdevice. First, the detection zone area (the plasma reservoir, Fig. 2a) is coated with the SARS-CoV-2 antigen (spike protein) by surface immobilization techniques (direct entrapment of antibodies by spotting and drying methods/physical absorption methods or plasma treatment) on the glass/PDMS (Heyries et al. 2007; Welch et al. 2017). Note that the glass surface is used for bonding purpose. Next, the PDMS-based microdevice is bonded onto the glass plate such that the plasma reservoir of the device aligns with the antigen-immobilized area. Next, BSA (bovine serum albumin) is added to prevent non-specific binding of antibodies. The device is now ready for the injection of whole blood. Blood is injected using a syringe pump delivering a constant flow rate of 0.5 ml/min; however, the use of expensive syringe pump can be avoided by devising a spring-loaded syringe capable of delivering the required flow rate. As blood flows into the microchannel, the plasma gets

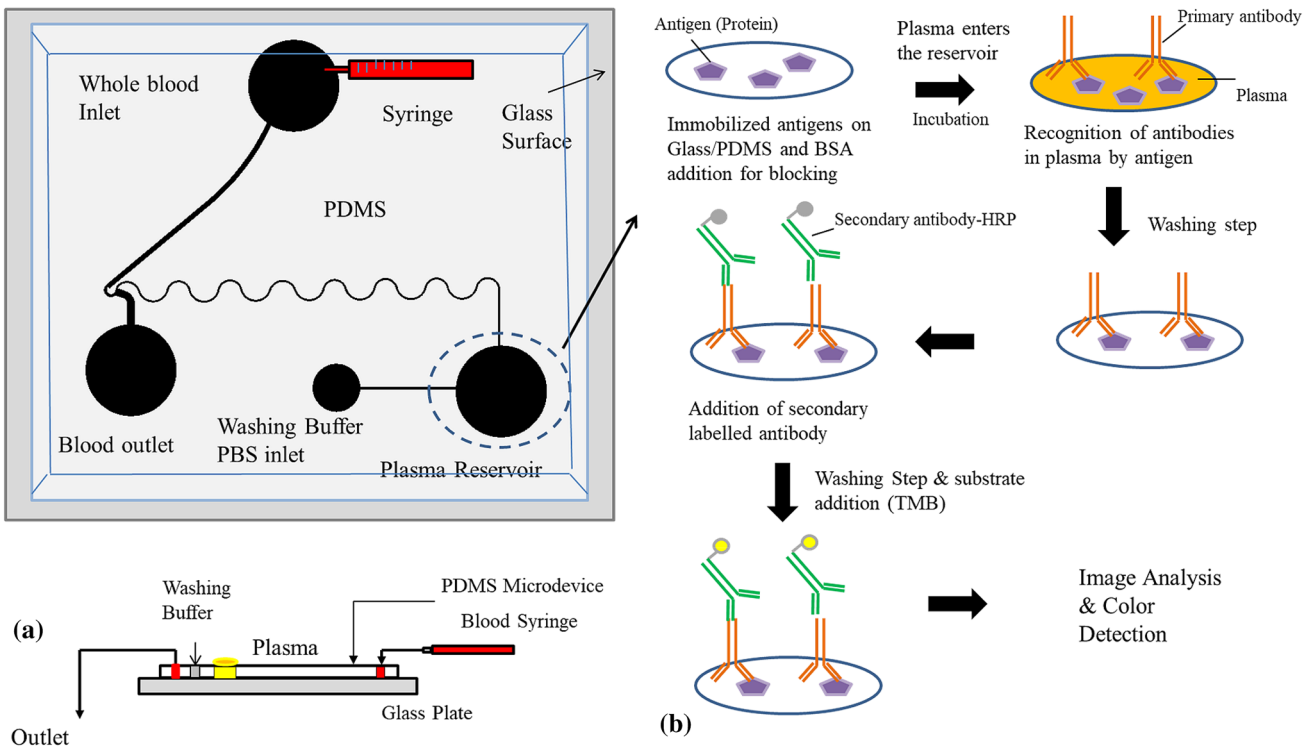

Fig. 2 **a** Top: mask of the original blood plasma separation microdevice design with additional inlet for carrying out washing steps and injecting antibodies. Bottom: side view of the microsystem. **b** Experimental sandwich ELISA: showing steps to identify the presence of SARS-CoV-2 (COVID-19) antibodies present in blood plasma

separated and flows towards the plasma outlet reservoir. The antibodies, or the target of interest binds to the SARS-CoV-2 antigens coated on the reservoir; this step involves reaction and incubation time. Subsequently, washing step is carried out by injecting PBS (phosphate-buffered saline and 0.1% Tween 20) from an additional reservoir connected through a channel near the plasma outlet. This step is essential to remove the unbound molecules. Next, labelled secondary antibodies (HRP—horseradish peroxidase conjugated) are injected and added to the reservoir. Finally, the substrate addition (TMB- 3,3′,5,5′-tetramethylbenzidine + H_2O_2) will result in colorimetric signals for image analysis and determining the concentration of COVID-19 antibody (Hsu et al. 2014). Though colorimetric methods are simple to employ, the electrochemical methods integrated with smartphone technology can also be employed for detection purposes (Lee and Lee 2013). Realizing a fully automated testing on a lab-on-chip format is quite challenging, it is expected that the proposed idea will be useful in reducing the sample processing and detection time as compared to conventional ELISA technique. In addition, the arrangement of the microdevice is such that other bio-markers of interest can also be measured simultaneously, if desired.

Timelines and Resources Envisaged

The development of the ELISA-based microfluidic platform will involve modifications in the current design and fabrication of the microdevice using photolithography and soft lithography techniques, plasma separation and off-chip testing of separated plasma using conventional 96-well ELISA, procurement of spike proteins, antibodies, buffers, bio-safety cabinet and spectrophotometer, immobilization of antigens and related experiments, quantification of antibodies (image analysis), and comparison of results obtained from the current microdevice with those obtained using a conventional ELISA kit. Overall, 12 months will be a reasonable estimate to accomplish the whole task.

Author Contributions Manuscript: ST; writing: ST; reviewing and editing the final manuscript: AA; writing the original draft: ST and AA; figure preparation: ST; resources: ST.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Petherick A (2020) Developing antibody tests for SARS-CoV-2. Lancet 395:1101–1102

Heyries KA, Marquette CA, Blum LJ (2007) Straightforward protein immobilization on Sylgard 184 PDMS microarray surface. Langmuir 23(8):4523–4527

Hsu CK, Huang HY, Chen WR, Nishie W, Ujiie H, Natsuga K, Fan ST, Wang HK, Lee JYY, Tsai WL, Shimizu H, Cheng CM (2014) Based ELISA for the detection of autoimmune antibodies in body fluid the case of bullous pemphigoid. Anal Chem 86(9):4605–4610

Lee J, Lee SH (2013) Lab on a chip for in situ diagnosis: from blood to point of care. Biomed Eng Lett 3(2):59–66

Liu D, Li X, Zhou J, Liu S, Tian T, Song Y, Zhu Z, Zhou L, Ji T, Yang C (2017) A fully integrated distance readout ELISA-Chip for point-of-care testing with sample-in-answer-out capability. Biosens Bioelectron 96:332–338

Prabhakar A, Kumar BV, Tripathi S, Agrawal A (2015) A novel, compact and efficient microchannel arrangement with multiple hydrodynamic effects for blood plasma separation. Microfluid Nanofluid 18(5–6):995–1006

Seo G, Lee G, Kim MJ, Baek SH, Choi M, Ku KB, Lee CS, Jun S, Park D, Kim HG, Kim SJ, Lee JO, Kim BT, Park EC, Kim SI (2020) Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano 14:5135–5142

Tripathi S, Prabhakar A, Kumar N, Singh SG, Agrawal A (2013) Blood plasma separation in elevated dimension T-shaped microchannel. Biomed Microdevice 15(3):415–425

Tripathi S, Kumar BV, Prabhakar A, Joshi SS, Agrawal A (2015a) Performance study of microfluidic device for blood plasma separation—a designer's perspective. J Micromech Microeng 25(8):084004

Tripathi S, Kumar BV, Prabhakar A, Joshi SS, Agrawal A (2015b) Passive blood plasma separation at the microscale: A review of design principles and microdevices. J Micromech Microeng 25(8):083001

Tripathi S, Kumar BV, Prabhakar A, Joshi SS, Agrawal A (2016) Microdevice for plasma separation from whole human blood using bio-physical and geometrical effects. Sci Rep 6:26749

Tripathi S, Varun Kumar YV, Agrawal A (2018) International Patent on Microdevice for separating plasma from human blood, Appl No. US Patent Application No 15/565,515

Vázquez-Guardado A, Barkam S, Peppler M, Biswas A, Dennis W, Das S, Seal S, Chanda D (2018) Enzyme-free plasmonic biosensor for direct detection of neurotransmitter dopamine from whole blood. Nano Lett 19(1):449–454

Vashist SK (2020) In vitro diagnostic assays for COVID-19: recent advances and emerging trends. Diagnostics 10:202

Yeh YT, Nisic M, Yu X, Xia Y, Zheng SY (2014) Point-of-care microdevices for blood plasma analysis in viral infectious diseases. Ann Biomed Eng 42(11):2333–2343

Welch NG, Scoble JA, Muir BW, Pigram PJ (2017) Orientation and characterization of immobilized antibodies for improved immunoassays. Biointerphases 12(2):02D301