University of Cincinnati

Date: 6/27/2013

I, Kang Kug Lee, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Electrical Engineering.

It is entitled:
A Sample-to-Answer Polymer Lab-on-a-Chip with Superhydrophilic Surfaces using a Spray Layer-by-Layer Nano-Assembly Method

Student's name: Kang Kug Lee

This work and its defense approved by:

Committee chair: Chong Ahn, Ph.D.
Committee member: Gregory Beaucage, Ph.D.
Committee member: Fred Beyette, Ph.D.
Committee member: Punit Boolchand, Ph.D.
Committee member: Jason Heikenfeld, Ph.D.
Committee member: Ian Papautsky, Ph.D.
A SAMPLE-TO-ANSWER POLYMER LAB-ON-A-CHIP WITH SUPERHYDROPHILIC SURFACES USING A SPRAY LAYER-BY-LAYER NANO-ASSEMBLY METHOD

A dissertation submitted to the

Division of Research and Advanced Studies of the University of Cincinnati

In partial fulfillment of the Requirements for the degree of

DOCTOR OF PHILOSOPHY (Ph. D.)

In the Department of Electrical Engineering and Computing Systems of the College of Engineering

June 27, 2013

By

Kang Kug Lee

M.S., Department of Mechanical Engineering, The Ohio State University, Columbus, OH, USA, 2005

M.S., Department of Material Science and Engineering, Hanyang University, Korea, 2000

B.S., Department of Material Science and Engineering, Hanyang University, Korea, 1992

Committee Chairman
Dr. Chong H. Ahn
ABSTRACT

In this research, an innovative ‘smart’ sample-to-answer (S-to-A) polymer lab-on-a-chip (LOC) platform with superhydrophilic surfaces has been proposed, developed, and fully characterized for point-of-care clinical testing (POCT) applications. A spray layer-by-layer (LbL) nano-assembly method has been developed and applied for the development of a superhydrophilic surface on a cyclic olefin copolymer (COC). Then, the developed superhydrophilic surfaces were designed and optimized for three device applications such as lateral transportation of whole blood in the device by capillary pumping, on-chip whole blood/plasma separation with an asymmetric capillary force, and detection using a capillary-driven lateral flow colorimetric assay. With the integration of the developed three devices, the S-to-A polymer LOC platform has been successfully developed for the calorimetric assay of bovine serum albumin (BSA) from a whole blood sample without an external power source.

One of the most important achievements in this research was to realize a new on-chip whole blood/plasma separator driven by asymmetric capillary forces. A nanoporous superhydrophilic surface by spray LbL nano-assembly method and a patterned hydrophobic patch were combined to attain asymmetric capillary forces through the microchannel for the effective separation of blood plasma from whole blood without external power resources. The blood plasma was effectively separated from the whole blood through the accumulated blood cells which worked as a so-called ‘self-built-in blood cell microfilter’. A spray LbL nano-assembly method developed for the superhydrophilic surfaces was simple, functional, practical, cost-effective, and a manufacturable tool for selectively constructing nanoporous multilayer surfaces at room
temperature. The resulting multilayer networks were effectively characterized by several characterization methods.

The newly developed superhydrophilic surfaces were also applied for the realization of a capillary-driven lateral flow colorimetric assay platform targeted to a S-to-A polymer LOC device. This superhydrophilic surface used for a capillary-driven lateral flow colorimetric assay has shown superior properties to the paper-based lateral flow colorimetric assay. Both high-quality blood plasma separated from whole blood and its quantitative analysis were simultaneously demonstrated in the S-to-A polymer LOC platform that was constructed by the integration of the on-chip blood plasma separator with the capillary-driven colorimetric assay. The limit of detection (LoD) in the S-to-A polymer LOC platform for BSA was comparable to or better than that of the paper-based colorimetric assay.

In conclusion, the ‘smart’ S-to-A polymer LOC device developed in this research is a generic platform for the POCT, particularly useful for physicians, nurses, patients in the developing world, in poor-resource settings, in the field, and in home-care settings because it is simple, disposable, inexpensive, and a user-friendly diagnostic device. The S-to-A polymer LOC platform is also envisaging a mass production for low-cost POCT applications.
To My Lovely Wife Mi Young

and My Three Lovely Daughters

Eun Jong (Sarah), Eun Se (Ashely), and In Ae (Rachel)
ACKNOWLEDGEMENTS

First of all, I would like to express deep appreciation to my Lord and Heavenly Father for guiding me in His perfect way during my Ph.D. study. He is always with me and gave me the great idea for the whole blood/plasma separation (Genesis 1:6~7) to complete my Ph.D. work. During this period, I have frequently read and kept in mind His Word, “Even though I walk through the valley of the shadow of death, I will fear no evil, for you are with me; your rod and your staff, they comfort me.” (Psalm 23:4)

I would also like to express great appreciation to my academic adviser, Dr. Chong H. Ahn for his valuable advice, extensive training, patience, and support toward my Ph.D. degree. He guided and led the way for me to make great discoveries in our research field. I have learned a lot from his strong and energetic passion for research as well as his Christian life. During my graduate studies, he has been a role model and mentor in my research and Christian life.

I would like to express my deep appreciation to Dr. Christian I. Hong for his valuable advice, kindness, and patience in performing his project, and support through the collaboration with Dr. Ahn. He helped to open the door in applying our device expertise in engineering to biological fields.

I would like to deeply thank all my committee members, Dr. Punit Boolchand, Dr. Fred Beyette, Dr. Jason Heikenfeld, Dr. Ian Papautsky, and Dr. Gregory Beaucage, who shared their expertise and experiences with me. Due to their valuable comments and critical questions about my work, I made discoveries I would not have expected and improved the quality of my dissertation. I would especially like to thank Dr. Ha Won Kim and Dr. Motoi Okada in the Department of Pathology and Laboratory Medicine at
the University of Cincinnati for procuring human whole blood samples. I also thank Dr. Paul Bishop and Dr. Am Jang for their support and valuable comments in performing NIH projects about potentiometric biosensors for environmental applications.

Additionally, I wish to appreciate the many people who enabled me to accomplish my Ph.D. work. Thanks to staff members in the engineering research center (ERC) clean room, Jeff Simkins, Ron Flenniken, and Dr. Robert Jones. They gave me technical assistance and training to achieve my Ph.D. work and projects. I also appreciate The Ohio Center for Microfluidic Innovation (OCMI) at the University of Cincinnati for partially supporting my work. In addition, I am very grateful to the former and current members in the BioMEMS and Microsystem Laboratory, University of Cincinnati. The former members include Dr. Jungyoup Han, Dr. Jaephil Do, Dr. Junhai Kai, Dr. Rong Rong, Dr. Sehwan Lee, Dr. Soohyun Lee, Dr. Chunyan Lee, Dr. Zhiwei Zou, Dr. Matthew Estes, Dr. Michael Rust, Dr. Pei-Ming Wu, Dr. Andrew Browne, Dr. Joon Sub Shim, Mr. Nathaniel Hadlock, Mr. Eric MacKnight, and Mr. Bharat Ram Ramaswamy. They provided me valuable technical advice, training, and technical know-how in our BioMEMS lab. And I want to thank my current lab members, Dr. Wooseok Jung, Zhizhen Wu, Nirjhar Bhattacharjee, Peng Wang, and Atreyee Bhattacharyya for their contributions to my work. I’d like to express my special thanks to Rev. Sung Kwon Lee and all the church members of The Power Mission Baptist Church of Cincinnati for their unyielding prayers and encouragement. Also, special thanks to my brother Dr. Seokheun Choi for his great help and discussion during my research. Another special thanks to Rev. Keun Sang Lee of The Korean Church of Columbus as a spiritual mentor. I am also very thankful for my brother
Paul Keel who sincerely prayed for me and helped me in proofreading of my publications. I also thank to my friend Paul Keates for his proofreading of my papers.

Finally, I would like to express my love and deep appreciation to my wife Mi Young and my three lovely daughters Eun Jong, Eun Se (Ashely), and In Ae (Rachel). I could not have completed my Ph.D. work without their care, love, support, and dedication. Mi Young usually prepared a delicious lunch box for me, which always reminded me her care and love that was a strong driving force to keep me moving forward with a great hope and passion for the successful achievement of my research work. They always trusted in Jesus Christ so I was able to focus on my Ph.D. study.
And God said, “Let there be an expanse between the waters to separate water from water.” So God made the expanse and separated the water under the expanse from the water above it. And it was so. (Genesis 1:6–7)
# TABLE OF CONTENTS

Table of Contents 1

List of Figures 4

List of Tables 13

Chapter 1. Introduction 14

1.1 Research Motivation 15

1.2 Review of Previous Work 18

1.3 Research Objectives 24

Chapter 2. Development of Superhydrophilic Surfaces on Cyclic Olefin Copolymer (COC) using Spray Layer-by-Layer (LbL) Nano-Assembly Method 27

2.1 Introduction 28

2.2 Experimental Procedures 35

2.2.1 Materials 35

2.2.2 A Spray Layer-by-Layer Nano-Assembly Method 35

2.2.3 Characterization Methods for the Nano-Assembled Silica Films 37

2.3 Experimental Results and Discussion 39

2.4 Conclusion 47
Chapter 3. Superhydrophilic Microchannels on COC by Spray LbL Nano-Assembly

Method

3.1 Introduction 49

3.2 Experimental Procedures 57

3.2.1 Design and Fabrication of COC Microchannels 57

3.2.2 Characterization of the Modified COC Microchannels 60

3.3 Experimental Results and Discussion 61

3.4 Conclusion 68

Chapter 4. A New On-chip Whole Blood/Plasma Separator using Asymmetric Capillary Forces

Capillary Forces

4.1 Introduction 70

4.2 Principle of Separation 77

4.3 Design and Fabrication 78

4.3.1 Microfabrication of On-chip Whole Blood/Plasma Separator 78

4.3.2 Spray LbL Nano-Assembly for On-chip Whole Blood/Plasma Separator 80

4.4 Experimental Results and Discussion 81

4.5 Conclusion 96

Chapter 5. A Whole Blood Sample-to-Answer (S-to-A) Polymer Lab-on-a-Chip (LOC) with Superhydrophilic Surfaces

5.1 Introduction 98
5.2 Design and Fabrication 106
  5.2.1 Microfabrication of S-to-A Polymer LOC Platform 106
  5.2.2 Preparation of Colorimetric Assay for Protein Quantification 109
5.3 Results and Discussion 111
5.4 Conclusion 119

Chapter 6. Conclusions 120
  6.1 Summary 121
  6.2 Research Contributions 125
  6.3 Suggestions for Future Work 126

References 127

Publications 139
LIST OF FIGURES

Figure 1.1 Constituents of the human whole blood.

Figure 1.2 An example of an ideal POC diagnostic device, which does not exist but toward the realization of a POC device in the near future. Images adapted from [20].

Figure 1.3 An example of a S-to-A LOC platform; an integrated blood barcode chip (IBBC) with on-chip plasma separation from a finger prick of blood using the Zweifach-Fung effect; multiple DNA-encoded antibody barcode arrays are patterned within the plasma-scanning channels for in situ measurement of plasma proteins using fluorescence detection. Images adapted from [21].

Figure 1.4 An example of a S-to-A LOC platform, which is a self-powered integrated microfluidic blood analysis system (SIMBAS); sample volume metering, on-chip plasma separation from undiluted whole blood, multiple immunoassays, and suction chambers for fluid transportation. Images adapted from [22].

Figure 1.5 An example of a S-to-A LOC platform; microfluidic paper-based analytical device with integrated blood plasma separation using a colorimetric assay. Images adapted from [23].

Figure 1.6 Conceptual diagram for a ‘smart’ S-to-A polymer LOC platform that simply integrates an on-chip blood plasma separator with a detection bioassay component without an external power source targeted to POCT applications.
**Figure 2.1** An example of a Langmuir-Blodgett (LB) technique; LB film is easily formed when a substrate is dipped into the interface; another monolayer of the LB films is formed with each insertion of the substrate. Images adapted from [30].

**Figure 2.2** An example of self-assembled monolayer (SAM); ideal SAM of alkanethiolates supported on a (111) gold surface; illustrating the intrinsic and extrinsic defects in SAMs on substrates. Images adapted from [32].

**Figure 2.3** An example of LbL nano-assembly method using slides and beakers; adsorption of polyanion and polycation with washing steps. Images adapted from [36].

**Figure 2.4** An example of processes used to fabricate polyelectrolyte multilayer formation by LbL nano-assembly; showing dipping, spin coating, and spraying LbL nano-assembly methods. Images adapted from [40].

**Figure 2.5** A spray LbL nano-assembly method: (a) the deposition of polyelectrolyte multilayer films on COC surfaces by spray LbL, where n is the number of bilayers and (b) spraying times and polyelectrolyte concentrations were based on the previous work [44], and no drying step was performed between layer depositions.

**Figure 2.6** Chemical structures of the polyelectrolytes in LbL nano assembly: (a) poly(diallyldimethylammonium chloride) and (b) poly(4-styrenesulfonic acid).

**Figure 2.7** Description of the contact angle measurements: (a) uncoated bare COC surface and (b) silica films on COC surface modified by spray LbL nano-assembly.
Figure 2.8 (a) Variation of contact angles on silica nanoparticle sizes with DI water droplets and (b) the weight difference between 1 bilayer and 5 bilayer coatings.

Figure 2.9 Contact angle variations as a function of time in different number of silica bilayers on COC surfaces.

Figure 2.10 Contact angle variations as a function of time with a droplet of whole blood, blood plasma, and DI water, respectively, on spray-coated silica multilayer surfaces.

Figure 2.11 AFM measurements: (a) model for AFM measurement; (b); (c); and (d) AFM images and RMS values of the spray nano-assembled silica nanoparticles on COC surfaces as a function of silica bilayers.

Figure 2.12 Long-term stability of the spray nano-assembled silica nanoparticle networks on COC surface.

Figure 3.1 An example of a passive capillary pump; the droplet radius $R$ will be larger than $h$ if the drop volume is less than that for the radius $r$; most of the blue dye was moved into the left vertical channel due to the capillary effect. Images adapted from [57].

Figure 3.2 An example of a passive capillary pump; this pump is designed to control the shape and orientation of the filling fronts; the pumping power of the pump relies on the contact angles of the filling liquid with its walls and the characteristic dimensions of its structures. Images adapted from [58].

Figure 3.3 An example of a passive capillary pump; showing degas-driven fluid flow in the vacuumed PDMS microfluidic device; the working principle is gas inside
the microchannel diffuses into the degassed PDMS; fluids can flow into the microchannels with the pressure difference, which is the driving force. Images adapted from [60].

**Figure 3.4** An example of a passive capillary pump; showing operation principle of the paper pump for pumping fluid in a microchannel; pumping of three different types of fluids with 60° sector paper pumps. Images adapted from [61].

**Figure 3.5** Schematic diagram showing nanoporous superhydrophilic multilayer silica nanoparticle networks developed onto a hydrophobic COC microchannel using a spray LbL electrostatic nano-assembly method.

**Figure 3.6** Computer numerical control (CNC) milling machine Microlution 5100-S (Microlution, USA) at The Ohio Center for Microfluidic Innovation (OCMI).

**Figure 3.7** Nano-assembly protocol for (a) the deposition of polyelectrolyte multilayer films on patterned COC microchannels by a spray LbL, where \( n \) is the number of bilayers; (b) spraying times and polyelectrolyte concentrations were based on the previous work [44], and no drying step was performed between layer depositions.

**Figure 3.8** An embossing machine (MTP-10, Tetrahedron Associates Inc., San Diego, CA) for thermoplastic fusion bonding at the Microsystems and BioMEMS lab.

**Figure 3.9** ESEM images of the (a) silica nanoparticle networks deposited onto COC microchannel and (b) bare COC microchannel.

**Figure 3.10** EDS spectra of the (a) silica nanoparticle networks deposited onto COC microchannel and (b) bare COC microchannel.
**Figure 3.11** Flow characterization in on-chip horizontal capillary pump with COC microchannels of 400μm x 100μm x 47 mm.

**Figure 3.12** Characterization of pumping capability in on-chip horizontal capillary pump with COC microchannels of 200 and 400 μm wide.

**Figure 3.13** On-chip blood plasma separation using a horizontal capillary pump with a closed 200 μm wide COC microchannel: (a) coating strategy of sidewall coatings only; (b) blood plasma separator with a closed COC microchannel; and (c) magnified view of the separated plasma.

**Figure 4.1** An example of an on-chip blood plasma separator using Zweifach–Fung effect; shows a tendency of red blood cells to travel into a daughter vessel with higher flow rate. Images adapted from [85].

**Figure 4.2** An example of an on-chip blood plasma separator by CD-type platform for centrifugation; proper control of the rotational speed allowed the blood plasma to be successfully separated in this centrifugal, microfluidic ‘lab-on-a-disk’ platform. Images adapted from [88].

**Figure 4.3** An example of on-chip blood plasma separator using hetero-packed beads at the inlet of a microchannel; two different sizes of silica beads were simply implemented as an integrated microfilter for plasma separation. Images adapted from [99].

**Figure 4.4** An example of an on-chip blood plasma separator by cross-flow filtration devices (Snake chip and U-Filter chip); shows filtration principle with different colors of arrows. Images adapted from [101].
**Figure 4.5** On-chip whole blood/plasma separator: (a) top view and (b) schematic side view.

**Figure 4.6** Summary of fabrication process of the on-chip whole blood/plasma separator: (a) fabrication procedure; (b) spray LbL nano-assembly; (c) device design; and (d) fabricated device.

**Figure 4.7** Variations of (a) contact angles and (b) capillary forces as a function of time in bare and spray modified COC surfaces.

**Figure 4.8** Coating strategies: (a) top wall coating only; (b) top wall coating with a 10 mm hydrophobic patch; (c) side wall coatings only; and (d) magnified view of the separated plasma.

**Figure 4.9** Separation results: (a) on-chip whole blood/plasma separator with 10 mm hydrophobic patch and (b) separation images over time.

**Figure 4.10** Schematic diagram for the volume of separated blood plasma from whole blood (side view); S1 is the leading edge of the separated blood plasma and S2 is the leading edge of the blood cells.

**Figure 4.11** Volume of the separated plasma (nL) as (a) the separation time (s); (b) microchannel width (µm) at 5 mm hydrophobic patch; (c) microchannel width (µm) at 10 mm hydrophobic patch; and (d) microchannel width (µm) at 35 mm hydrophobic region.

**Figure 4.12** Captured images to determine the threshold concentration for percolation of RBC's across the microchannel.

**Figure 4.13** Measurements of the length of blood cells in hydrophobic patch region in terms of the whole blood dilution with PBS solution. The threshold
concentration was obtained in 50X dilution based on the Figure 4.12. The measurements were performed in five times.

**Figure 5.1** An example of the colorimetric assay results on chromatography paper. The colorimetric assays for glucose and protein are demonstrated using various concentrations of glucose and BSA. Images adapted from [132].

**Figure 5.2** An example of a colorimetric assay and the results of a digitized intensity diagram for both grayscale and cyan channels; the display engine is Adobe Photoshop. This result is then communicated to physicians located off-site. This figure shows the procedure for quantifying the levels of glucose and protein in urine. The concentration of the analyte can be obtained from the mean pixel values within the test zones. Images adapted from [133].

**Figure 5.3** An example of colorimetric assays on paper-based microfluidic devices for the simultaneous semi-quantitative analysis of glucose, lactate, and uric acid. Images adapted from [138].

**Figure 5.4** An example of a paper-based microfluidic chip with the colorimetric detection of salivary nitrite. Color intensities were quantified using a flatbed scanner and image manipulation software and plotted against concentration to produce calibration curves for the assay. Images adapted from [139].

**Figure 5.5** Working principle of a S-to-A polymer LOC device with capillary-driven lateral flow colorimetric assay platform: (a) chip fabrication with spray LbL nano-assembly; (b) spotting reagent for colorimetric assay and whole blood injection; (c) on-chip blood plasma separation; and (d) color change due to the lateral flow colorimetric assay.
**Figure 5.6** Summary of fabrication process of the S-to-A polymer LOC device: (a) fabrication procedures; (b) spray LbL nano-assembly method; (c) device design; and (d) fabricated device.

**Figure 5.7** Preparation of colorimetric assay for protein quantification in a S-to-A polymer LOC platform: (a) a S-to-A polymer LOC device; (b) spotting the citrate buffer solution followed by TBPB in ethanol for colorimetric assay and drying for 10 min; (c) injection of the spiked whole blood into the device; and (d) color change due to the colorimetric protein assay.

**Figure 5.8** Capillary-driven lateral flow colorimetric assay in a S-to-A polymer LOC platform: (a) spotting the layered 0.3 μL of 3.3 mM of tetrabromophenol blue (TBPB) in 95% ethanol over the 0.3 μL of 500 mM citrate buffer solution (pH 2.5) onto the superhydrophilic COC surfaces; (b) protein detection assays by using various concentrations of bovine serum albumin (BSA); and (c) magnified view of the separated blood plasma in a 400 μm width microchannel.

**Figure 5.9** Volume of the separated plasma (nL) over time in the S-to-A polymer LOC platform; 400 μm width microchannel with a 10 mm hydrophobic patch. The volume of the separated plasma below 300 seconds was slightly increased over time due to the accumulation of blood cells over the hydrophobic patch during the separation. The volume of the separated plasma was rapidly increased after 300 seconds due to strong asymmetric capillary force throughout the ‘self-built-in blood cell microfilter’ developed in a 10 mm hydrophobic patch.
**Figure 5.10** Procedure for quantifying protein levels in separated blood plasma through a S-to-A polymer LOC device using Adobe Photoshop software: (a) colorimetric assay by using various concentrations of BSA; (b) conversion of photographed digital images to grayscale mode; and (c) a mean value within the selected test zone.

**Figure 5.11** Quantification of protein concentration in human whole blood samples using a S-to-A polymer LOC device with integrated on-chip blood plasma separator. The colorimetric assay was calibrated with blood plasma samples separated from three microchannels with known protein concentrations ranging from 0 mg/mL to 5 mg/mL (0 μM to 75 μM) of BSA. The limit of detection was 0.025 mg/mL (25μg/mL).
**LIST OF TABLES**

**Table 4.1** Effects of the length of hydrophobic patch on the separation results of the blood plasma.

**Table 4.2** Effects of the width of microchannel on the separation results of the blood plasma.
CHAPTER 1

INTRODUCTION
1.1 **Research Motivation**

Human whole blood is one of the key biological fluids sampled from the human body and used for clinical diagnostics. As blood is circulating through the body every minute, it carries oxygen and nutrients to cells, removes waste, regulates temperature and pH, exchanges products through tissues, and supports the immune system. Therefore, whole blood is a good indicative for inspecting the body at the molecular level because blood contains a massive amount of critical information concerning the function of the whole body [1]. Many diagnostic tests are performed on blood plasma separated from human whole blood because changes in its composition reflect the current status of the pathological processes throughout the body [2].

Human whole blood is mainly composed of cellular components and plasma as shown in Figure 1.1. The cellular components, 45% of blood volume, consist of erythrocytes (red blood cells), leukocytes (white blood cells), reticulocytes, and thrombocytes (platelets) [3, 4]. The remaining 55% of blood volume is plasma which contains proteins (albumin, globulins, clotting factors, etc.), electrolytes, antibodies, antigens, and hormones. Plasma acts as a Newtonian fluid with a constant viscosity, however, the red blood cells in cellular components are responsible for the non-Newtonian behavior [5, 6]. Serum corresponds to the plasma with blood clotting factors removed.

A blood test is the most common medical diagnostic tool to monitor the health condition of patients who have chronic diseases (such as diabetes) and is of great importance in global health applications. This is because faster or more efficient treatment can be delivered due to the rapid and precise diagnosis of diseases with the
whole blood that contain critical information concerning the function of the body as mentioned above. A blood test is also the best clinical diagnostic for urgent care patients in regions with poor resources, in the field, or in combat zones. Typical blood tests are performed in a central laboratory which is equipped with bulky and expensive analyzers, and usually require a considerable volume of blood sample, trained personnel, and need relatively long analysis time [7]. Furthermore, the separation of blood plasma from the whole blood is a critical step for most clinical blood analysis based on fluorescence immunoassays to minimize the noise from blood cells interfered with excitation optics. These limitations can be removed with the development of more practical blood analytical systems.

**Figure 1.1** Constituents of the human whole blood.
The microfluidic LOC platform has been developed for the various fields in chemistry, biology, engineering, and biomedical and pharmaceutical research over the past decades [8–14]. With the recent development of microfluidic LOC technology, the miniaturization of laboratory instruments and assays has been realized, leading to lower costs per measurement, reduced sample analysis times, reduced sample volume, and better reproducibility in several applications. Compared with the 96-well based microtiter plate, the use of a microchip reduces sample consumption from ~100 µL to ~1 µL. More importantly, the assay time can also be reduced from several hours to several minutes. The reduction of assay time is mainly due to the short diffusion length that the molecules must travel to immobilize on the solid phase surface because the cross-sectional dimension of a microchannel is around 10 µm to 100 µm [15].

However, most of the previously reported LOC platforms have limitations in their use because of the need for an external power source in fluid handling such as high-voltage power supplies for the electrokinetic flow, pressure sources such as syringe pumps for the pressure-driven flow, and a mechanical rotator for the centrifugation induced flow [16–19]. These instruments are often bulky and expensive, and restrict the use of microchips as an integrated on-chip platform. Furthermore, at a central laboratory equipped with these instruments, the total turnaround time (TAT) from sample-to-result is extended by the time required for the transportation of sample to the central laboratory. Due to these limitations, this research has proposed and explored an innovative ‘smart’ S-to-A polymer LOC platform that simply integrates a miniaturized on-chip blood plasma separator with a capillary-driven lateral flow bioassay component without an external power source. This device concept demonstrates that results are obtained after the
injection of a sample onto the platform with no further processing described in Figure 1.2, which is an ideal POC diagnostic device.

**Figure 1.2** An example of an ideal POC diagnostic device, which does not exist but toward the realization of a POC device in the near future. Images adapted from [20].

### 1.2 REVIEW OF PREVIOUS WORK

Recently, only a few groups have implemented the automated S-to-A LOC platform that integrates an on-chip blood plasma separator with a bioassay-based detection element for POCT applications. Heath’s group at Caltech developed an integrated blood barcode chip (IBBC) for the rapid measurement of plasma proteins from a finger prick of whole blood [21] (Figure 1.3). Integration of a microfluidic system with a DNA-encoded barcode chip enabled a reliable on-chip blood separation (using Zweifach-Fung effect) and *in situ* measurement of plasma proteins within 10 minutes. This time scale is short enough to evade most protein degradation which occurs in
sampled blood. The developed IBBC is a potential device for point-of-care settings because the device is a minimally invasive, low-cost, and an informative clinical diagnostic platform. The IBBC can be a good example of the S-to-A LOC platforms for POCT applications.

**Figure 1.3** An example of a S-to-A LOC platform; an integrated blood barcode chip (IBBC) with on-chip plasma separation from a finger prick of blood using the Zweifach-Fung effect; multiple DNA-encoded antibody barcode arrays are patterned within the plasma-scanning channels for *in situ* measurement of plasma proteins using fluorescence detection. Images adapted from [21].

Lee’s group at the University of California, Berkeley demonstrated a self-powered integrated microfluidic blood analysis system (SIMBAS), which efficiently separates blood plasma from a 5 μL droplet of undiluted whole blood followed by multiple protein binding assays [22] (Figure 1.4). For on-chip blood plasma separation, blood cells are separated from undiluted whole blood by trapping them in an integral filter trench structure. This integrated device does not require any external networks or tubing to
transport and analyze the introduced whole blood sample within 10 minutes because of the degas-driven flow powered by pre-evacuation of its PDMS substrate. The developed SIMBAS is very suitable for POCT applications because it is a high-throughput, robust, portable, low-cost, low sample volume, simple-to-use disposable platform. Furthermore, the objective of the SIMBAS concept for POC molecular diagnostics is to minimize the number of elements while achieving the self-powered, tubing-free single-chip integration critical for maximum efficiency in performing bioassays. The developed SIMBAS concept is another good example of a S-to-A LOC platform for POCT applications.

Figure 1.4 An example of a S-to-A LOC platform, which is a self-powered integrated microfluidic blood analysis system (SIMBAS); sample volume metering, on-chip plasma separation from undiluted whole blood, multiple immunoassays, and suction chambers for fluid transportation. Images adapted from [22].
Shevkoplyas’ group at Tulane University designed and fabricated a microfluidic paper-based analytical device (μPAD) with integrated blood plasma separation [23] (Figure 1.5). The blood plasma was separated from finger-prick volumes of whole blood due to the red blood cell (RBC) agglutination in the paper-fluidic platform. By integrating
plasma separation with RBC agglutination and colorimetric assay into a single μPAD platform, a paper-based S-to-A LOC platform was realized in this work. The developed device is composed of a blood plasma separation zone and four test readout zones (Figure 1.5). The patterning of the μPAD is simply performed on chromatography paper using a wax printer. The μPAD was functionalized by spotting agglutinating antibodies onto the plasma separation zone in the center and the reagents of the colorimetric assay onto the test readout zones on the periphery of the device. When a droplet of whole blood is injected into the center of the device, agglutinated RBCs remain in the central zone while separated plasma was transported into the test readout zones. In the test readout zone, a visual color change was produced due to the reaction of the analyte in separated blood plasma with the reagents of the colorimetric assay. A calibration curve was generated through the digitization of the color change and the conversion to concentration values using a portable scanner. The colorimetric assay for plasma glucose with small samples of whole blood was successfully performed in a μPAD because of a high purity and a high yield of the separated blood plasma. With the developed separation approach, a fully integrated S-to-A LOC platform using small samples of whole blood will be mainly useful in resource-limited settings for POCT applications.

As described above, there is still a large demand for the development of an innovative ‘smart’ S-to-A polymer LOC platform (Figure 1.6) that simply integrates an on-chip blood plasma separator with a detection bioassay component without an external power source targeted to POCT applications.
Figure 1.6 Conceptual diagram for a ‘smart’ S-to-A polymer LOC platform that simply integrates an on-chip blood plasma separator with a detection bioassay component without an external power source targeted to POCT applications.
1.3 **Research Objectives**

The objective of this research is to develop an innovative ‘smart’ S-to-A polymer LOC platform that simply integrates an on-chip whole blood/plasma separator with a capillary-driven lateral flow colorimetric assay component without an external power source targeted to POCT applications. The developed superhydrophilic surface by a spray LbL nano-assembly method was designed for three specific goals; lateral transportation of whole blood in the device by capillary pumping, on-chip whole blood/plasma separation with an asymmetric capillary force, and detection using a capillary-driven lateral flow colorimetric assay. The developed S-to-A polymer device is an ideal platform for POCT applications, particularly useful in poor-resource settings because it is simple, disposable, low-cost, and user-interactive diagnostic device.

To achieve this research objective, four major research tasks have been performed:

1. Development and characterization of superhydrophilic surfaces on COC by spray LbL nano-assembly method.
2. Development and characterization of superhydrophilic microchannels on COC by spray LbL nano-assembly method.
3. Development and characterization of a new on-chip whole blood/plasma separator using asymmetric capillary forces.
4. Development and characterization of a S-to-A polymer LOC platform with superhydrophilic surface toward POCT applications.

Each of the desired research tasks to be performed is addressed in the following chapters. Chapter 2 focuses on the development and characterization of superhydrophilic surfaces on COC by a spray LbL nano-assembly method. The nanocapillary-driven
superhydrophilic multilayer networks with 12 nm silica nanoparticles onto the COC surfaces is successfully prepared by a spray LbL nano-assembly method. The resulting multilayer networks are successfully characterized by several characterization methods.

Chapter 3 presents nanocapillary-driven superhydrophilic multilayer networks with 12 nm silica nanoparticles onto the patterned COC microchannels which are successfully prepared by a spray LbL nano-assembly method. The resulting multilayer networks on COC microchannels are successfully characterized by two characterization methods and two testing platforms. The on-chip capillary micropump drives the food dye 47 mm without any additional power within 12 seconds. The same platform with closed microchannels for on-chip blood plasma separation presents about a 10 nL volume of blood plasma which is successfully separated from the whole blood.

Chapter 4 discusses the fabrication and characterization of an innovative asymmetric capillary force driven on-chip whole blood/plasma separator. A nanoporous superhydrophilic surface by spray LbL nano-assembly method and a patterned 10 mm hydrophobic patch are combined to attain asymmetric capillary forces through the microchannel for the separation of blood plasma from whole blood. The blood plasma separator developed in this work successfully separates blood plasma from undiluted human whole blood without an external power source. This separator with a simple asymmetric microchannel can be flexibly integrated into a ‘smart’ S-to-A polymer LOC device for POCT applications.

Chapter 5 presents a ‘smart’ S-to-A polymer LOC platform that successfully integrates an on-chip blood plasma separator with capillary-driven lateral flow colorimetric assay component without an external power source. The developed S-to-A
polymer LOC platform is favorable for mass production and commercialization because the thermoplastic COC used for the device fabrication is a good candidate for mass production by injection molding or hot embossing processes.

Finally, this thesis concludes with a summary and discussion of this work as well as the research contributions and suggestions for future work in Chapter 6.
CHAPTER 2

DEVELOPMENT OF SUPERHYDROPHILIC SURFACES ON CYCLIC OLEFIN COPOLYMER USING SPRAY LAYER-BY-LAYER NANO-ASSEMBLY METHOD
2.1 INTRODUCTION

Controlling and/or modifying the surface wettability of solid substrates is important for a fundamental understanding of surface structure and behavior in many applications [24, 25]. Both superhydrophilic (water contact angle less than 5°) and superhydrophobic (water contact angle greater than 150°) surfaces are particularly desirable for various practical applications [25]. Considerable efforts have been made to obtain superhydrophilic surfaces in various applications such as self-cleaning, antifogging, enhancement of boiling heat transfer, and for household appliances [26–29]. However, much less investigation has been reported to use superhydrophilic surfaces in microfluidic and biomedical devices, where the surfaces are usually in direct contact with DNA, proteins, or cells. It is well known that cell adhesion and protein adsorption onto a substrate are highly sensitive to the wettability and the chemical nature of the surface.

Various techniques for ultrathin film preparation have been reported widely such as the Langmuir-Blodgett (LB) technique [30, 31] (Figure 2.1) and the self-assembled monolayer (SAM) method [32, 33] (Figure 2.2). Unfortunately, these approaches are not always useful for practical application because they require rather expensive and special instruments, harsh processing conditions, high cost and ineffective method for multilayer fabrication. Compared with these traditional approaches, the electrostatic LbL nano-assembly method is a simple, inexpensive, and versatile process for building functional nanostructures using a sequential approach to defining multilayer ultrathin films and allows a variety of materials to be incorporated in the film structures [34–43]. The LbL nano-assembly method, first reported by Iler [39] in 1966 and then rediscovered by Decher et al. [34, 35] in the early 1990s, has recently become a general method for
alternately depositing monolayers of charged molecules onto oppositely charged surfaces [34]. This approach is extremely useful because of the generality of the process, which depends mainly on the attractive force between oppositely charged molecules.

**Figure 2.1** An example of a Langmuir-Blodgett (LB) technique; LB film is easily formed when a substrate is dipped into the interface; another monolayer of the LB films is formed with each insertion of the substrate. Images adapted from [30].
Figure 2.2 An example of self-assembled monolayer (SAM); ideal SAM of alkanethiolates supported on a (111) gold surface; illustrating the intrinsic and extrinsic defects in SAMs on substrates. Images adapted from [32].

Materials used in this nano-assembly range from charged polymers [36–39], proteins [37, 38], nanoparticles [39–41], dyes [40–42] and clay nanoplates [37–43]. Each layer has a thickness on the order of a few nanometers, and this thickness can be controlled through careful selection of materials and reaction parameters [37]. Furthermore, the composition of films can be tailored through the sequential deposition
of different materials. Complex film architecture may be achieved through a common process without the need for complicated chemistry. Therefore, the LbL nano-assembly method provides a practical procedure for building precisely engineered nanocomposite films.

The LbL nano-assembly is commonly carried out by the dipping method [34–36] (Figure 2.3). Negatively charged substrate is immersed into an aqueous solution of polycation. After a specific time for the layer to adsorb on the substrate, the sample is rinsed with rinsing solution to remove weakly bound polycation molecules in order to avoid their bulk reaction with polyanions, which could happen during the following adsorption step. After rinsing, the monolayer film is obtained. Because of the long period of time required to assemble a single film (e.g., 1 min to 1 hr depending on adsorbing systems, typically 10–20 min), the procedure is time-consuming and inconvenient for routine sample preparation. Another disadvantage is the difficulty in application of multilayers to large surface areas. In an attempt to overcome some of these problems, alternative methods for the application of multilayers using spraying and spinning approaches have been developed [40–43] (Figure 2.4). These approaches need to develop a novel method to modify the hydrophobic polymer surfaces as well.
Figure 2.3 An example of LbL nano-assembly method using slides and beakers; adsorption of polyanion and polycation with washing steps. Images adapted from [36].
Figure 2.4 An example of processes used to fabricate polyelectrolyte multilayer formation by LbL nano-assembly; showing dipping, spin coating, and spraying LbL nano-assembly methods. Images adapted from [40].

In this work, a spray LbL nano-assembly method combining the LbL method and the spraying technique has been proposed and developed for modifying the COC polymer surfaces to be superhydrophilic described in Figure 2.5. This spray LbL method is a simple, low cost, versatile, and high-throughput process for the multilayer formation at room temperature. The major advantage of this method is a dramatically reduced processing time needed for the multilayer formation at room temperature. Surface functionality can be easily controlled by choosing appropriate polyelectrolytes.
Figure 2.5 A spray LbL nano-assembly method: (a) the deposition of polyelectrolyte multilayer films on COC surfaces by spray LbL, where $n$ is the number of bilayers and (b) spraying times and polyelectrolyte concentrations were based on the previous work [44], and no drying step was performed between layer depositions.
2.2 EXPERIMENTAL PROCEDURES

2.2.1 Materials

Two kinds of polyelectrolyte, polydimethylallyl-ammonium chloride (PDDA) and polysodium 4-styrenesulfonate (PSS) as an adhesion promoter were purchased from Sigma-Aldrich (St. Louis, MO). The chemical structures of the polyelectrolyte used are depicted in Figure 2.6. As hydrophilic nanoparticles with different sizes, SM-30 colloidal silica 30 wt % (7 nm diameter silica particles), Ludox HS-40 colloidal silica 40 wt % (12 nm diameter silica particles), and Ludox TM-40 colloidal silica 40 wt % (22 nm diameter silica particles) were also purchased from the Sigma-Aldrich. In addition, the other silica nanoparticles (5 % aqueous dispersion, 50 nm and 100 nm diameter silica particles) used for the nano-assembly were obtained from Polysciences (Warrington, PA). All negatively charged colloidal silica nanoparticles were diluted to 0.3 wt % in deionized (DI) water (18.2 MΩcm). Polyelectrolyte solutions (PDDA and PSS) were diluted to 0.01 M in DI water. A blank bare COC (TOPAS Advanced Polymers Inc., KY) wafer was cut into 15 mm × 15 mm rectangular shapes for the experiment. All rectangular COCs were cleaned by immersion in methanol and acetone solutions followed by a rinsing in DI water and dried with high-pressure high-purity nitrogen gas.

2.2.2 A Spray Layer-by-Layer Nano-Assembly Method

Figure 2.5 shows a schematic diagram of a typical multilayer buildup process by a spray LbL nano-assembly method [44–46]. The polyion solution and rinsing solution were supplied by enforced spraying in this process. The surface was modified by successive spraying of polyelectrolytes (PDDA or PSS or colloidal silica nanoparticles)
on the COC microchannel surfaces, which were already activated by oxygen plasma (MARCH CS-1701 RIE, March Instruments Inc., CA) shown in Figure 2.5(a). The film was spray-rinsed with DI water after a complete layer was formed. Spray rinsing was suspended but drainage and evaporation of water continue. Then, the layer of oppositely charged polyon was sprayed onto the spray-rinsed surface displayed in Figure 2.5(b). The total processing time per layer was just one minute which was a dramatically reduced processing time needed for the multilayer formation at room temperature. By simply choosing suitable polyelectrolytes in the spray LbL nano-assembly, the surface functionality was easily organized as desired.

**Figure 2.6** Chemical structures of the polyelectrolytes in LbL nano assembly: (a) poly(diallyldimethylammonium chloride) and (b) poly(4-styrenesulfonic acid).
2.2.3 Characterization Methods for the Nano-Assembled Silica Films

The contact angle analysis is a simple but very powerful method for measuring the changes of surfaces at the monolayer level. The wettability of the spray-coated silica films on COC was characterized in ambient air at room temperature using a contact angle goniometer based on the sessile drop method described in Figure 2.7. The mean contact angles with high purity DI water were determined by averaging values measured at five different points on the sample surface. The atomic force microscopy (AFM) images were obtained by Veeco Dimension 3100 AFM (Digital Instruments Inc., Santa Barbara, CA) under ambient conditions. Cantilevers with a spring constant of 0.03 N/m and silicon nitride tips were used. Several scans were performed over a given surface area. These scans were aimed at producing reproducible images to ascertain that there was no sample damage induced by the tip. Deflection and height mode images were scanned simultaneously at a fixed scan rate (between 2 and 4 Hz) with a resolution of 512 × 512 pixels.
Figure 2.7 Description of the contact angle measurements: (a) uncoated bare COC surface and (b) silica films on COC surface modified by spray LbL nano-assembly.


2.3 EXPERIMENTAL RESULTS AND DISCUSSION

2.3.1 Surface Characterization with Contact Angle Measurements

Figure 2.8(a) shows that the contact angles on the modified COC surfaces were measured as a function of five types of silica nanoparticles. The lowest contact angle with the smallest standard deviation was obtained in 12 nm silica nanoparticles coated onto the COC surface. Therefore, the 12 nm silica nanoparticle was chosen for the spray LbL nano-assembly on the COC microchannels as the surface control applications such as microfluidic capillary pump or separator due to the strong hydrophilic nature of the multilayer nanoporous structure.

The contact angle variation with nanoparticle sizes was analyzed by hemi-wicking wetting [47, 48], the dynamics of a droplet imbibing on a rough surface. A critical contact angle $\theta_c$ is defined as below:

$$\theta < \theta_c, \text{ with } \cos \theta_c = \frac{1-\phi_s}{r-\theta_s}$$

(1)

The roughness factor $r$ is the ratio of the actual surface area over the projected one, and $\phi_s$ is the solid fraction of the projected area covered by the top of the roughness. For the porous materials, the liquid can be infiltrated between hollows induced by the capillary force, so the solid can be regarded as textured structures. While a liquid droplet is placed on the rough surface satisfying $\theta < \theta_c$ in Equation (1), infiltration of the liquid into the hollows of the rough surface is energetically favorable. Equation (1) exhibits the intermediate condition between for spreading and for imbibition. In other words, the condition generally states a critical contact angle intermediate between 0 and $\pi/2$ if the $r$ > 1 and $\phi_s < 1$. Spreading on a surface occurs if $\theta_c \rightarrow 0$ for a flat surface ($r \rightarrow 1$), however, the classical condition for capillary rise is satisfied when $\theta_c \rightarrow \pi/2$ in porous
media ($r \to \infty$). Based on the dynamics of capillary imbibition, a smaller contact angle was obtained in the smaller silica nanoparticles deposited on COC compared with the larger silica nanoparticles on COC. Below 10 nm scale, however, this hemi-wicking wetting cannot be properly applied because the nanoparticles are not simply assembled onto the COC surface. For the sizes between 1 and 10 nanometers with a narrow size distribution, the nanostructures can be classified into nanoclusters [49]. Nano-sized particles are usually flying away from the targeted substrates during spraying because their kinetic energy is sufficiently high. The amount of free flying particles was especially high in relatively smaller nanoparticles (1–10 nm) considering the weight difference between bilayers in Figure 2.8(b). It is noted that the weight difference between the bilayers in 7 nm was dramatically low, which presents a low deposition ratio compared with that in the other nanoparticles. The water contact angle of the surface assembled with silica nanoclusters of 7 nm was relatively high in about 18 degrees compared with that of 12 nm. Therefore, the minimum contact angle value in 12 nm silica nanoparticles was attributed to the dynamics of a capillary imbibition on LbL assembled nanoporous architectures as well as the safely assembled nanoparticles onto the COC surface.
Figure 2.8 (a) Variation of contact angles on silica nanoparticle sizes with DI water droplets and (b) the weight difference between 1 bilayer and 5 bilayer coatings.
Figure 2.9 Contact angle variations as a function of time in different number of silica bilayers on COC surfaces.

Figure 2.9 shows the changes in contact angle for a first water drop (3 μL) as a function of time. The contact angles of all surfaces were gradually decreased over time. Highly wettable surfaces were obtained with coatings of 5, 10 and 15 bilayers but superhydrophilic behavior was only achieved in a coating of 5 bilayers for the film that completely wets (contact angle below 5° compared to bare COC with a contact angle of 90°) with DI water after 250 seconds. This dramatic drop in contact angles indicated that the COC surface would be superhydrophilic with silica nanoparticles due to the
intrinsically high level of wettability of the silica nanoparticles coupled with the nanoporous nature of the multilayer surface. Thus, the coating of five [PDDA/silica] bilayers was chosen as an optimum coating of bilayers to obtain the maximum nanoporous capillary pumping effect in this process. Figure 2.10 shows the changes in contact angle as a function of time for a droplet (3 μL) of whole blood, blood plasma, and DI water, respectively, on spray-coated silica multilayer surfaces. A dramatic drop was obtained with DI water after 250 seconds due to intrinsically high level of wettability of the silica nanoparticles and nanoporous superhydrophilic nature of the multilayer surface.

**Figure 2.10** Contact angle variations as a function of time with a droplet of whole blood, blood plasma, and DI water, respectively, on spray-coated silica multilayer surfaces.
2.3.2 Surface Characterization with AFM

Figure 2.11 shows a model for AFM measurement, surface morphology, and roughness information of the spray nano-assembled silica networks obtained by AFM. The surface topography on the nanometer length scale was altered by simply varying the number of silica bilayers. Moreover, increasing the number of silica bilayers resulted in a corresponding increase in root-mean-square (RMS) roughness values. The five silica bilayers had smoother surfaces with a low RMS roughness of 4.83 nm in Figure 2.11(b) compared to the fifteen silica bilayers, which had an RMS roughness of 12.1 nm in Figure 2.11(d). Thus, the surface roughness value was increasing as the number of silica bilayers assembled by spray LbL nano-assembly method.

2.3.3 Long-term Stability of the Nano-Assembled Silica Nanoparticles on COC

The long-term stability of the deposited films is an important parameter for the evaluation of the film performance. The durability of the spray nano-assembled silica nanoparticles on COC surface was investigated by monitoring the contact angle variations over 60 days in Figure 2.12. The coated samples were kept in outdoor exposure during the periods at room temperature. The results showed that superhydrophilic coating by spray LbL nano-assembly method was chemically stable over time.
Figure 2.11 AFM measurements: (a) model for AFM measurement; (b); (c); and (d) AFM images and RMS values of the spray nano-assembled silica nanoparticles on COC surfaces as a function of silica bilayers.
Figure 2.12 Long-term stability of the spray nano-assembled silica nanoparticle networks on COC surface.
2.4 CONCLUSION

In conclusion, nanocapillary-driven superhydrophilic multilayer networks with 12 nm silica nanoparticles onto the COC surfaces was successfully prepared by a spray LbL nano-assembly method. The resulting multilayer networks were successfully characterized by several characterization methods. This dramatic drop in contact angles indicated that the COC surface would be superhydrophilic with silica nanoparticles due to the intrinsically high level of wettability of the silica nanoparticles coupled with the capillary imbibitions of the LbL assembled nanoporous architectures. AFM measurement showed the surface roughness value was increasing as the number of silica bilayers assembled by spray LbL nano-assembly method. Developed superhydrophilic coating by spray LbL nano-assembly method was chemically stable over time. Therefore, the developed superhydrophilic silica multilayer networks on COC surfaces using a spray LbL nano-assembly method can also be a useful and promising platform for on-chip whole blood/plasma separator and capillary-driven lateral flow bioassays targeted to POCT.
CHAPTER 3

SUPERHYDROPHILIC MICROCHANNELS

ON CYCLIC OLEFIN COPOLYMER BY SPRAY LAYER-BY-LAYER NANO-ASSEMBLY METHOD
3.1 **INTRODUCTION**

LOC, especially, microfluidics-based LOC technology has been considered a promising platform for a lot of applications during the past few decades [8–14]. The most important aspect within microfluidic LOC devices is probably handling and transport of fluids. Control of the fluids in microfluidics based LOC such as capillary micropump or separator can be usually achieved with either actuated or passive microfluidics [50–52]. Although the actuated microfluidics are capable of powerful pumping and transporting large volumes, there are a lot of limitations that are not suitable for POCT, including complicated sample-interface connections, a minimum requirement of sample volumes, very large dead volumes, and peripheral equipment that can restrict device portability [53–56]. Due to these restrictions, passive microfluidics with no external power source are more desirable for the most microfluidic based LOC devices toward POCT. Among some passive pumping methods [57–61], flow control of biological samples or reagents using capillary pumping through a microchannel is very attractive for the on-chip microfluidic devices of bioanalytical systems and POCT. Beebe’s group at University of Wisconsin-Madison showed a good example of a passive capillary pump [57] (Figure 3.1). The driving force for pumping through a microchannel was the existing surface energy in a small drop of liquid. In the side view of the microchannel, the droplet radius \( R \) will be larger than \( h \) if the drop volume is less than that for the radius \( r \). And most of the blue dye was moved into the left vertical channel due to the capillary effect. Delamarche’s group at IBM Research GmbH, Zurich Research Laboratory showed autonomous capillary systems [58] (Figure 3.2). This work demonstrated the design rule for capillary pumps to control the flow properties of the capillary systems. The design
rule was to control the placement and the shape of the microstructures in the capillary pumps for obtaining the reliable filling behavior. Contact angles of the filling liquid with its walls and the characteristic dimensions of its structures were an important role for controlling the pumping power of the pump.

Figure 3.1 An example of a passive capillary pump; the droplet radius $R$ will be larger than $h$ if the drop volume is less than that for the radius $r$; most of the blue dye was moved into the left vertical channel due to the capillary effect. Images adapted from [57].
Figure 3.2 An example of a passive capillary pump; this pump is designed to control the shape and orientation of the filling fronts; the pumping power of the pump relies on the contact angles of the filling liquid with its walls and the characteristic dimensions of its structures. Images adapted from [58].
Lee’s group at the University of California, Berkeley demonstrated a passive capillary pump with degas-driven fluid flow in polydimethylsiloxane (PDMS) microfluidic devices [60] (Figure 3.3). The gas inside the microchannel can diffuse into the degassed PDMS because of high porosity and air solubility of the PDMS. Thus, fluids can flow into the microchannels by the pressure difference without any external power. Papautsky’s group at University of Cincinnati showed a passive capillary pump with paper [61] (Figure 3.4). This work demonstrated the programmable flow-rate control during the pumping process by using the paper-based pumps. They tried to combine the advantages of fluid transport by paper and functional microchannels by polymer into a simple LOC platform for POCT.

**Figure 3.3** An example of a passive capillary pump; showing degas-driven fluid flow in the vacuumed PDMS microfluidic device; the working principle is gas inside the microchannel diffuses into the degassed PDMS; fluids can flow into the microchannels with the pressure difference, which is the driving force. Images adapted from [60].
Figure 3.4 An example of a passive capillary pump; showing operation principle of the paper pump for pumping fluid in a microchannel; pumping of three different types of fluids with 60° sector paper pumps. Images adapted from [61].
Polymer-based microfluidic LOC devices are an alternative to those fabricated from glass and silicon substrates along with the standard lithographic microfabrication technology [62]. Polymers are suitable for single-use disposable devices since they offer low cost, good biocompatibility and mass manufacture using high-throughput microfabrication techniques such as hot embossing or injection molding. Various polymer materials [63–69] such as polycarbonate (PC), polyvinyl chloride (PVC), polymethylmethacrylate (PMMA), polystyrene (PS), and COC have been used for the microfluidic LOC devices. Among them, a thermoplastic COC is of particular interest due to its combination of excellent UV transparency, low autofluorescence, low oxygen permeability, high mechanical strength, and compatibility with a broad range of chemicals and solvents [70]. However, like most other polymers, COC has a hydrophobic nature, which causes many problems in the applications of biochip or LOC such as sample loss, degradation of resolution in separations, and difficulties with accurate quantitative analyses. In order to minimize these problems, it is necessary to modify or functionalize the walls of the COC microchannels. As already mentioned in Chapter 2, various techniques of polymer surface modification have been developed and reported in several reviews [71–75]. However, most of these procedures are not suitable for microfluidic devices because they rarely allow selective patterning on the surface of the microchannel. Furthermore, they require additional special instruments, harsh processing conditions, and high cost. Therefore, there is still a large demand for the development of a new technique for attaining highly hydrophilic surfaces on the patterned polymer microchannels toward a passive microfluidic capillary pump.
In this work, a passive on-chip capillary pump with nanoporous superhydrophilic multilayer silica nanoparticle networks has been developed onto a hydrophobic COC microchannel using a spray LbL electrostatic nano-assembly method shown in Figure 3.5. This modification method is a powerful yet facile, practical, easy-to-use (non-trained personnel), and high-throughput for selectively constructing nanoporous media onto the hydrophobic polymer surfaces. The major advantage of this spray LbL is a dramatically reduced processing time needed for the multilayer formation, specifically, on the thermoplastic COC at room temperature. Surface functionality can be easily controlled by choosing appropriate polyelectrolytes. The pumping capability of the on-chip micropump is achieved from the strong hydrophilic properties of the nanoporous multi-coated bilayers of sprayed silica nanoparticles. The asymmetric capillary force induced between the nanoporous superhydrophilic surfaces and the uncoated hydrophobic COC surface offered a strong driving force for separating the blood plasma from the human whole blood in a closed microchannel. These properties were mainly due to the nanoporous infiltration multilayer structures onto the COC microchannels.
Figure 3.5 Schematic diagram showing nanoporous superhydrophilic multilayer silica nanoparticle networks developed onto a hydrophobic COC microchannel using a spray LbL electrostatic nano-assembly method.
3.2 EXPERIMENTAL PROCEDURES

3.2.1 Design and Fabrication of COC Microchannels

A computer numerical control (CNC) milling machine (Microlution 5100-S, Microlution Inc., IL) shown in Figure 3.6 was selected as a method for fabricating a master mold. The CNC milling manufactures various sizes of structures from micrometer-scale to millimeter-scale with high precision and high reliability. The three main steps for the CNC milling were computer-aided design (CAD), computer-aided manufacturing (CAM) programs, and a CNC machine for production. In this work, the straight microchannels with the dimensions of 200 and 400 μm width × 100 μm depth × 50 mm length were designed with the program AutoCAD (Autodesk Inc., CA). With the solid model in Mastercam (CNC Software Inc., CT) CAM, the two-dimensional CAD drawing was converted to a three-dimensional drawing. Then, the tool-paths were produced based on the three-dimensional structural information using the solid model. The created tool-paths were converted to specific computer codes that were recognized by the Microlution 5100-S CNC machine and the CNC fabricated the device. After the procedure of CNC milling, the master mold containing straight microchannels was fabricated on the 6061 aluminum alloy with a 4 inch width, 4 inch length, and 0.25 inch thickness for the replica of COC microchannels. Then, COC substrates were patterned with microfluidic channels using a hot embossing technique for the following spray LbL nano-assembly process.

Figure 3.7 shows a schematic diagram of a typical multilayer build-up process for the superhydrophilic surface by the spray LbL nano-assembly method mentioned in Chapter 2. The polyion solutions and rinsing solutions were supplied by enforced
spraying in this process. The surface was modified by successive spraying of polyelectrolytes (PDDA or PSS or colloidal silica nanoparticles) on the COC microchannel surfaces, which were already activated by oxygen plasma (MARCH CS-1701 RIE, March Instruments Inc., CA) shown in Figure 3.7(a). The film was spray-rinsed with DI water after a complete layer was formed. Spray rinsing was suspended but drainage and evaporation of water continue. Then, the layer of oppositely charged polyanion was sprayed onto the spray-rinsed surface displayed in Figure 3.7(b). The total processing time per layer was just one minute which was a dramatically reduced processing time needed for the multilayer formation at room temperature. By simply choosing suitable polyelectrolytes in the spray LbL nano-assembly, the surface functionality was easily organized as desired. Finally, the polymer substrate with modified microchannels was bonded with a cover polymer substrate by a thermoplastic fusion bonding technique [75] using an embossing machine (MTP-10, Tetrahedron Associates Inc., San Diego, CA) shown in Figure 3.8.

**Figure 3.6** Computer numerical control (CNC) milling machine Microlution 5100-S (Microlution, USA) at The Ohio Center for Microfluidic Innovation (OCMI).
Figure 3.7 Nano-assembly protocol for (a) the deposition of polyelectrolyte multilayer films on patterned COC microchannels by a spray LbL, where n is the number of bilayers; (b) spraying times and polyelectrolyte concentrations were based on the previous work [44], and no drying step was performed between layer depositions.
Figure 3.8 An embossing machine (MTP-10, Tetrahedron Associates Inc., San Diego, CA) for thermoplastic fusion bonding at Microsystems and BioMEMS lab.

3.2.2 Characterization of the Modified COC Microchannels

The images of the COC microchannel and the film formed by silica nanoparticles were characterized by ESEM (environmental scanning electron microscopy, XL-30, Philips-FEI Company, Holland). Energy dispersive X-ray spectroscopy (EDS) analysis was performed over the microchannel area with and without the silica nanoparticles to confirm the spray-assembled silica nanoparticles. The capillary effect of the developed surface was characterized by measuring the length of a test liquid driven by the induced capillary forces in an on-chip capillary pumping platform with horizontal microchannels. The developed networks with spray-assembled nanoparticles were also applied for an on-chip blood plasma separation platform with closed microchannels. A sample of whole human blood was procured from the department of Pathology and Laboratory Medicine at the University of Cincinnati.
3.3 **Experimental Results and Discussion**

3.3.1 Characterization of COC Microchannels with ESEM and EDS

Nano-assembled silica film onto COC microchannel and the uncoated COC microchannel were characterized by ESEM as shown in Figure 3.9. A high resolution image of the silica nanoparticles onto the COC microchannel was depicted in Figure 3.9(a). This close and uniform nanostructure observed in ESEM image enhanced the nanoporous capillary action of the deposited silica nanoparticles. The enhanced nanoporous capillary force was attributed to the hydrophilic nature of the nano-assembled silica nanoparticles on a hydrophobic COC surface as well as the imbibition into porous architecture discussed in Chapter 2. In order to confirm the silica nano-assembly, EDS analysis was performed over the COC microchannel areas with and without the silica nanoparticles as shown in Figure 3.10. The chemical signature of silicon and oxygen peaks in EDS spectra were displayed within the COC microchannel area with the coated silica nanoparticles in Figure 3.10(a). These signatures clearly presented that the nanostructure of silica nanoparticles was successfully constructed onto the COC microchannel by the spray LbL nano-assembly method.
Figure 3.9 ESEM images of the (a) silica nanoparticle networks deposited onto COC microchannel and (b) bare COC microchannel.
Figure 3.10 EDS spectra of the (a) silica nanoparticle networks deposited onto COC microchannel and (b) bare COC microchannel.
3.3.2 Flow Characterization in Microfluidic Capillary Pump

Two types of microchannels, 200 and 400 μm wide in a horizontal platform were prepared to characterize the nanoporous capillary pumping effect. All microchannel surfaces were coated with 5 [PDDA/silica] bilayers. A 3 μL droplet of red-colored food dye was injected into the inlet of the microchannel using a micropipette. A flow was produced through the microchannel to reach the outlet reservoir by an autonomous capillary. As shown in Figure 3.11, the liquid front of the food dye moved up to 47 mm without any additional forces within 12 seconds. It was found that the liquid moved quickly at the beginning time and the speed of the movement slightly decreased at the succeeding time until the liquid reached the outlet reservoir. Figure 3.12 shows the relationship between the relative distance of liquid-gas interface locations from inlet to outlet reservoirs and time. The liquid front location of the food dye moved approximately 42 mm for the initial seven seconds and moved 5 mm during the succeeding three seconds when the width of the microchannel was 200 μm. As the width was 400 μm, the liquid-gas interface location could only move 37 mm for the first seven seconds but moved 6 mm during the succeeding three seconds. Thus, the capillary pumping effect was decreasing with the increasing width of the microchannels.
Figure 3.11 Flow characterization in on-chip horizontal capillary pump with COC microchannels of 400$\mu$m x 100$\mu$m x 47 mm.

Figure 3.12 Characterization of pumping capability in on-chip horizontal capillary pump with COC microchannels of 200 and 400 $\mu$m wide.
3.3.3 On-chip Blood Plasma Separation in a Closed COC Microchannel

Figure 3.13 displays the on-chip blood plasma separation in a closed COC microchannel. The blood cells were not allowed to move forward because of the viscosity difference among the whole blood. However, the blood plasma was separated from the whole blood due to a strong asymmetric capillary force [76] developed between a plain hydrophobic COC surface and three nanoporous superhydrophilic surfaces although the microchannel was closed in the capillary pump shown in Figure 3.13(b). About a 10 nL volume of the separated plasma was finally obtained in the blood plasma separator depicted in Figure 3.13(c). This platform is a good example of the passive on-chip capillary pump with nanoporous superhydrophilic silica multilayers onto hydrophobic COC microchannel using a spray LbL electrostatic nano-assembly method. It is very desirable to generate a strong asymmetric capillary force among the surfaces for separating the blood plasma from whole blood in a closed microchannel.
Figure 3.13 On-chip blood plasma separation using a horizontal capillary pump with a closed 200 μm wide COC microchannel: (a) coating strategy of sidewall coatings only; (b) blood plasma separator with a closed COC microchannel; and (c) magnified view of the separated plasma.
3.4 CONCLUSION

In conclusion, nanocapillary-driven superhydrophilic multilayer networks with 12 nm silica nanoparticles onto the patterned COC microchannels was developed by a spray LbL nano-assembly method. Then, the resulting multilayer networks on COC microchannels were successfully characterized by two characterization methods and two testing platforms. An ESEM image showed the close and uniform nanostructure, which enhanced the nanoporous capillary action of the deposited silica nanoparticles. The chemical signatures in EDS spectra clearly showed that the nanostructure of silica nanoparticles was successfully constructed onto the COC microchannel by the spray LbL nano-assembly method. The result also showed that the on-chip capillary micropump drives the food dye 47 mm without any additional power within 12 seconds. The capillary pumping effect was decreasing with the increasing width of the microchannels. The same platform with closed microchannels for on-chip blood plasma separation presented that about 10 nL volume of blood plasma was successfully separated from the whole blood. This separation result was due to the strong asymmetric capillary force developed between a plain hydrophobic COC surface and the three nanoporous superhydrophilic surfaces in spite of the closed microchannels in the platform. Therefore, the developed superhydrophilic silica multilayer networks on microchannels using a spray LbL nano-assembly method can also be a practically useful platform for on-chip whole blood/plasma separator and capillary-driven lateral flow bioassays targeted to POCT.
CHAPTER 4

A NEW ON-CHIP WHOLE BLOOD/PLASMA SEPARATOR USING ASYMMETRIC CAPILLARY FORCES
4.1 INTRODUCTION

A blood test is one of the most common diagnostic methods to evaluate health conditions for patients because human whole blood contains critical information concerning the function of the whole body [1]. Typical blood tests are performed in a central laboratory which is equipped with bulky and expensive analyzers, and usually require a considerable volume of blood sample, trained personnel, and need relatively long analysis time [7]. Furthermore, the separation of blood plasma from the whole blood is a critical step for most clinical blood analysis based on fluorescence immunoassays to minimize the noise from blood cells interfered with excitation optics. With the impact of microfluidic LOC technology [11–14], there is a large demand for the development of an on-chip blood plasma separator easily integrated into a ‘smart’ S-to-A microfluidic LOC platform for blood testing shown in Chapter 1.

Various on-chip blood plasma separators have been reported, utilizing geometrical obstruction [77–79], cross-flow filtration [80–82], bifurcation law [83–85], CD type platform for centrifugation [86–88], dielectrophoretic (DEP) forces [89–91], magnetic force [92, 93], acoustic force [94, 95], membrane filtration [96–98], and microfilters [99–101]. Yang et al. at Pennsylvania State University showed blood plasma separation using bifurcation law (Zweifach-Fung effect) [85] (Figure 4.1). However, the separation based on these devices usually required complicated fabrication processes and precise fluidic control. The device also needs external mechanical pumping force such as a syringe pump or other fluidic pumps to drive the liquid into microchannels. These external pumping elements cannot be easily integrated into the S-to-A microfluidic LOC devices. Haeberle et al. at University of Freiburg showed another type of blood plasma
separation by CD-type platform for centrifugation [88] (Figure 4.2). Proper control of the rotational speed allowed the blood plasma to be successfully separated in this centrifugal, microfluidic ‘lab-on-a-disk’ platform. However, this platform was difficult to integrate with other functional protocols such as polymerase chain reaction for nucleic acid amplification.

**Figure 4.1** An example of an on-chip blood plasma separator using Zweifach–Fung effect; shows a tendency of red blood cells to travel into a daughter vessel with higher flow rate. Images adapted from [85].
Figure 4.2 An example of an on-chip blood plasma separator by CD-type platform for centrifugation; proper control of the rotational speed allowed the blood plasma to be successfully separated in this centrifugal, microfluidic ‘lab-on-a-disk’ platform. Images adapted from [88].

Ahn’s group at University of Cincinnati demonstrated a simple on-chip blood plasma separator using hetero-packed beads at the inlet of a microchannel [99] (Figure 4.3). Two different sizes of silica beads were easily implemented as an integrated microfilter for plasma separation. This bead packed microfilter allowed capillary separation of plasma from the whole blood. Sollier et al. at CEA-LETI-Minatec showed passive microfluidic filtration devices for the plasma separation from whole human blood.
[101] (Figure 4.4). Two kinds of devices such as a Snake chip and a U-Filter chip were fabricated and used for the cross-flow filtration. However, these devices were only efficient for a short period of time due to fast cell clogging. The clogged blood cells were difficult to be practically removed from the device.

Figure 4.3 An example of on-chip blood plasma separator using hetero-packed beads at the inlet of a microchannel; two different sizes of silica beads were simply implemented as an integrated microfilter for plasma separation. Images adapted from [99].
Figure 4.4 An example of an on-chip blood plasma separator by cross-flow filtration devices (Snake chip and U-Filter chip); shows filtration principle with different colors of arrows. Images adapted from [101].

As previously mentioned, most of these separators have limitations in their use such as a use of high diluted blood, clogging, complexity in fabrication and operation, and slow speed. Furthermore, some of them are impractical because they need external power sources for fluid handling, not suitable for on-chip applications toward POCT. Therefore, it is very desirable to develop a more practical on-chip blood plasma separator
easily integrated into the ‘smart’ S-to-A microfluidic LOC platform for better separation capability of blood plasma from undiluted human whole blood without any external power source toward POCT.

In this work, an innovative asymmetric capillary force driven on-chip whole blood/plasma separator, which can be integrated into the S-to-A microfluidic LOC platform, has been proposed and developed for POCT. This separator is very useful for the POCT platform because it is simple, self-powered, tubing-free, and filter-free within the device. Since the separator is made of COC, a mass production is possible using injection molding or a hot embossing process. A spray LbL nano-assembly method has been first applied for the surface modification of naturally hydrophobic COC to be superhydrophilic. This spray LbL nano-assembly is a practical, fast, and easy-to-apply modification method for most thermoplastic surfaces at room temperature. The blood plasma separator operates by utilizing both the asymmetric capillary force and the patterned hydrophobic patch without any external power resources and is able to efficiently separate enough plasma from a single drop (3 µL) of whole blood which is a suitable amount for the single-use application of POCT. This small amount of whole blood is important because separating an effective amount of blood plasma from a small amount of whole blood is very useful for neonates, pediatrics, resource-limited environments, and home-care settings for chronic disease.
Figure 4.5 On-chip whole blood/plasma separator: (a) top view and (b) schematic side view.
4.2 **Principle of Separation**

The basic principle of the on-chip whole blood/plasma separator is based on the asymmetric capillary microchannel with a patterned hydrophobic patch as described in Figure 4.5. When human whole blood was introduced into the inlet of the separator, the whole blood was moving fast due to the capillary force produced from the silica-assembled top wall. However, the flow of the whole blood was effectively impeded in the middle of the microchannel due to the patterned hydrophobic patch which prevented the flow of blood cells. The hydrophobic patch as a flow barrier caused the accumulation of blood cells over the patch. The accumulated column of blood cells worked as a built-in microfilter, and the blood plasma was successfully separated from the whole blood throughout this ‘self-built-in blood cell microfilter’. Since the viscosities between blood cells and plasma were fairly different, the blood plasma from the whole blood in the microchannel was rapidly flowing along the superhydrophilic surface. Thus, the blood plasma was flowing first across the hydrophobic patch and then towards the other microchannel region which was composed of both the nanoporous superhydrophilic surface and the plain hydrophobic surface. Since a strong asymmetric capillary force through the microchannel was developed, the blood plasma was successfully separated from the whole blood.
4.3 DESIGN AND FABRICATION

4.3.1 Microfabrication of On-chip Whole Blood/Plasma Separator

Figure 4.6(a) summarizes the fabrication process of the blood plasma separator. A CNC milling machine was used for fabricating a master mold. The CNC milling machine manufactures various sizes of structures in the range of μm to mm in scale. In this work, the microchannel was designed to measure the separated volume of plasma from whole blood and to characterize the asymmetric capillary movement of blood. The volume of the inlet reservoir was approximately 3 μL, which was comparable to the volume of whole blood taken from a finger prick utilizing commercial devices. A microchannel in the dimension of 100 μm width × 100 μm depth × 50 mm length and the spray coated surface with a 10 mm hydrophobic patch was designed for the on-chip whole blood/plasma separator.

The master mold containing straight microchannels was fabricated on the 6061 aluminum alloy with a 4 inch width, 4 inch length, and a 0.25 inch thickness for the replica of COC microchannels. Then, COC substrates were patterned with microfluidic channels using a hot embossing technique for the spray LbL process. Then, the spray LbL nano-assembly process with silica nanoparticles [44] was performed for the asymmetric superhydrophilic surface with 10 mm hydrophobic patch over the COC microchannel. Finally, the COC substrate with microchannels was bonded with the spray modified COC substrate by a thermoplastic fusion bonding technique, which is applying high temperature and pressure, using an embossing machine [75]. The designed microchannel with 100 μm × 100 μm × 50 mm and a 10 mm hydrophobic patch are shown in Figure 4.6(c). The fabricated on-chip whole blood/plasma separator is shown in Figure 4.6(d).
Figure 4.6 Summary of fabrication process of the on-chip whole blood/plasma separator:
(a) fabrication procedure; (b) spray LbL nano-assembly; (c) device design; and (d) fabricated device.
4.3.2 Spray LbL Nano-assembly for On-chip Whole Blood/Plasma Separator

A typical multilayer build-up process by spray LbL is schematically described in Figure 4.6(b) indicating the desired processing times (total 1min per cycle) for each process. A COC was prepared to fabricate the microfluidic blood plasma separator. The COC surface, activated by oxygen plasma, was modified by successive spraying of PDDA and PSS as an adhesion promoter. These polymer solutions were diluted to 0.01 M in DI water. Rinsing step with DI water was performed right after a complete layer was formed. Then, the layer of oppositely charged polyions and rinsing solution were supplied by enforced spraying in this process shown in Figure 4.6(a). Ludox HS-40 colloidal silica 40 wt % (12 nm diameter silica particles, Sigma-Aldrich), negatively charged and diluted to 0.3 wt % in DI water, was used for the spray LbL nano-assembly. The PDDA and silica nanoparticles were applied to the COC surface by spraying. Spraying was suspended but drainage and evaporation of water continue. The deposited film was also spray-rinsed with DI water.
4.4 Experimental Results and Discussion

The wettability of the spray coated surfaces of COC was characterized in ambient air at room temperature using a contact angle analyzer based on the sessile drop method (Chapter 2) that is a simple, but very powerful method for measuring the changes of surfaces at the monolayer level. The mean contact angles with high purity DI water were determined by averaging values measured at five different points on the sample surface. 2 µL of DI water was applied on the sample surfaces using a micropipette. As shown in Figure 4.7(a), the radical drop in contact angles by the spray LbL nano-assembly method [44] indicated that the COC surface would be superhydrophilic (contact angle below 5° compared to bare COC with a contact angle of 88°) with silica nanoparticles. The surfaces showed the high level of wettability of silica nanoparticles which were coupled with the nanoporous nature of the multilayer surface. Five coatings of (PDDA/silica) bilayers were chosen as the optimum coatings of bilayers in order to obtain the maximum nanoporous capillary pumping effect in this process. 12 nm silica nanoparticles were adopted for the spray LbL nano-assembly on the COC surfaces since the lowest contact angle with the smallest standard deviation was obtained from the COC surface coated with that size [44].

The capillary forces induced by the spray LbL nano-assembly method can be calculated in terms of the measured contact angles based on the equation (1) [34] below.

\[ F_{\text{cap}} = 4\pi\gamma R \cos \theta = 4\pi(72.8 \text{ mJ/m}^2)(6) \cos \theta \approx 5.5 \cos \theta \ (nN) \]  

(1)

, where \(\gamma\) is the surface tension of water at 20 °C (72.8 mJ/m²), \(R\) is the radius of the nanoparticle, and the \(\theta\) is the contact angle. From this calculation, the larger the capillary force is, the more the hydrophilic surface is. Thus, maximum capillary force was obtained
in the lowest contact angle, which represented the superhydrophilic nature of the surface shown in Figure 4.7(a). Asymmetric capillary force can be defined by the difference of the capillary forces induced between the hydrophilic and hydrophobic surfaces based on the equation (2) below.

\[ F_{\text{cap, asymmetric}} = F_{\text{cap, hydrophilic}} - F_{\text{cap, hydrophobic}} \]

\[ \approx 5.5 \left( \cos \theta_{\text{hydrophilic}} - \cos \theta_{\text{hydrophobic}} \right) (\text{nN}) \]  (2)

From the equation (2), a strong asymmetric capillary force would be induced between the hydrophobic COC surface and the spray LbL assembled superhydrophilic COC surface. This asymmetric capillary force would be used as a strong driving force for the separation of mixed constituents such as human whole blood.
Figure 4.7 Variations of (a) contact angles and (b) capillary forces as a function of time in bare and spray modified COC surfaces.
Figure 4.8 described three different types of coating strategies onto the cover COC or patterned COC microchannels in an on-chip whole blood/plasma separator. The silica nanoparticles by spray LbL nano-assembly method were deposited onto the COC surfaces to obtain the asymmetric superhydrophilic surfaces for achievable blood plasma separations. Figure 4.8(a) showed the separator only coated on the top wall in the microchannel. Figure 4.8(c) showed the separator coated on side walls and bottom along the microchannel. When whole blood was injected into the inlet of the separator, the blood movement was driven by the capillary force induced by the coated top wall in Figure 4.8(a) or the coated side and bottom walls in Figure 4.8(c). Since these structures did not provide enough retarding force required for the separation of blood plasma even if the asymmetric capillary forces along the microchannel surfaces moved the whole blood, only a fairly small amount of separation was obtained as shown in Figure 4.8(a) and (c). However, a large enough amount of blood plasma separation for the POCT was achieved by utilizing both the asymmetric capillary force and the 10 mm hydrophobic patch as shown in Figure 4.8(b). This figure showed that the patterned hydrophobic patch was an essential component for achieving the blood plasma separation from whole blood as described in Figure 4.5. Figure 4.8(d) showed the magnified clear image of the separated blood plasma that represented the high quality of filtering efficiency by asymmetric capillary force and the patterned hydrophobic patch.

With the device structure described in Figure 4.8(b), the sequential results of the separated plasma from the whole blood were depicted in Figure 4.9. When human whole blood was dropped into the inlet of the blood plasma separator, the top superhydrophilic surface allowed for the capillary flow of whole blood through the microchannel. When
whole blood encountered the 10 mm hydrophobic patch region during this movement, the flow of the whole blood was effectively retarded for a short period of time (< 1 min).

Figure 4.8 Coating strategies: (a) top wall coating only; (b) top wall coating with a 10 mm hydrophobic patch; (c) side wall coatings only; and (d) magnified view of the separated plasma.
Figure 4.9 Separation results: (a) on-chip whole blood/plasma separator with 10 mm hydrophobic patch and (b) separation images over time.

This retarding effect caused continuous accumulation of the blood cells within the hydrophobic patch region. The movement of the blood cells was effectively reduced, allowing the blood plasma to move forward due to the differences in flow velocity between the cellular component and the blood plasma over the patch region. Thus, the
blood plasma was successfully separated from the whole blood throughout this accumulated column of blood cells, so-called ‘self-built-in blood cell microfilter’.

All samples of human whole blood used in this work were procured and used for the blood plasma separator based on the previous procedure [99, 102, 103] in the Department of Pathology and Laboratory Medicine at the University of Cincinnati. Some blood samples from several healthy volunteers were obtained with the help of Dr. Ha Won Kim and Dr. Motoi Okada (specialist for blood sampling) in the Department of Pathology and Laboratory Medicine. All blood samples were contained in a BD Vacutainer® Plus Plastic K$_2$EDTA Tube (green top tube, Becton & Dickinson Company, NJ) because the green top is the plasma tube used for blood plasma separation [104]. In addition, the blood plasma separation in the device was performed within 2 hours of blood collection because the purpose of this research is to develop a blood plasma separator for the on-site monitoring system with a single droplet of 3 μL human whole blood, enough for a disposable single-use platform for POCT. Blood samples drawn from different volunteers were successfully separated as shown in Figure 4.8 and Figure 4.9.

By using reference marks along with the microchannel, the movement of the separated plasma was monitored with an optical microscope. The volume of separated plasma was obtained by measuring the length of the leading edges between separated blood plasma and blood cells over time to characterize the separation performance described in Figure 4.10 and equation (3) below.

\[
\text{The volume of separated blood plasma} = \\
\{(\text{Leading edge of the separated blood plasma: } S1) - (\text{Leading edge of the blood cells: } S2)\} \times (\text{channel width}) \times (\text{channel depth})
\]
Figure 4.10 Schematic diagram for the volume of separated blood plasma from whole blood (side view); S1 is the leading edge of the separated blood plasma and S2 is the leading edge of the blood cells.

The blood plasma was successfully separated through the 10.2 mm long microchannel, where its cross-sectional area was 100 μm x 100 μm. The volume of the separated plasma was large enough for on-chip POCT with undiluted human whole blood. A volume of 102 nL of the separated plasma from a drop of whole blood was finally obtained as shown in Figure 4.11(a). The volume of the separated plasma between 300 and 400 seconds in Figure 4.11(a) was slightly decreased over time due to the accumulation of the blood cells over the hydrophobic patch during the separation. Successful separation was obtained only from the top coating with the 10 mm hydrophobic patch as shown in Figure 4.11(a). To optimize the width of the patterned hydrophobic patch in the separator, whole blood was injected and monitored in the microchannels with different sizes of the widths and the hydrophobic patches. It is noted that capillary force or the moving tendency in the leading edges of the blood cells and the
separated plasma is influenced by the length of the hydrophobic patch shown in Table 4.1.

**Figure 4.11** Volume of the separated plasma (nL) as (a) the separation time (s); (b) microchannel width (μm) at 5 mm hydrophobic patch; (c) microchannel width (μm) at 10 mm hydrophobic patch; and (d) microchannel width (μm) at 35 mm hydrophobic region.

The blood cells were retarded yet were still moving slower than the blood plasma in the hydrophobic patch region. In a 5 mm hydrophobic patch that is not long enough to
retard blood cells, the capillary force was strong enough in the leading edges of blood cells and separated plasma.

<table>
<thead>
<tr>
<th>Hydrophobic patch length (mm)</th>
<th>Capillary force in the leading edge</th>
<th>Side views of the separator (100 μm channel width)</th>
<th>Top views of the separator (100 μm channel width)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5</td>
<td>Strong</td>
<td><img src="image" alt="Side views of the separator" /></td>
<td><img src="image" alt="Top views of the separator" /></td>
</tr>
<tr>
<td>10</td>
<td>Strong</td>
<td><img src="image" alt="Side views of the separator" /></td>
<td><img src="image" alt="Top views of the separator" /></td>
</tr>
<tr>
<td>35</td>
<td>Weak</td>
<td><img src="image" alt="Side views of the separator" /></td>
<td><img src="image" alt="Top views of the separator" /></td>
</tr>
</tbody>
</table>

Table 4.1 Effects of the length of hydrophobic patch on the separation results of the blood plasma.

The blood cells were bridging across the patch and were not accumulated in the region, so a fairly small amount of blood plasma was separated from the whole blood. Figure 4.11(b) showed the volume variation of the separated plasma as four different microchannel widths with a 5 mm hydrophobic patch. Over 6 nL volumes were obtained in the widths of the 100 and 400 μm microchannels, however, those were not enough volume for the POCT. In a 35 mm hydrophobic patch, the capillary forces were weak.
enough in the leading edges of blood cells and separated plasma due to no asymmetric capillary force at the other side of the patch region. Thus, the flow of both components was fairly slow and only a small amount of blood plasma was separated. The volume variation of the separated plasma as four different microchannel widths with a 35 mm hydrophobic region was described in Figure 4.11(d). A 16 nL volume of the separated plasma was obtained in the 400 μm microchannel width of the separator, which is still not enough volume for the POCT. In the microchannel with 10 mm hydrophobic patch, the capillary force in the leading edge of the separated plasma was strong enough but that of the blood cells was quite weak. So, the blood cells were effectively retarded during the separation of the blood plasma. In Figure 4.11(c), the highest volume of the separated blood plasma 102 nL, a suitable amount for disposable LOC platform toward POCT, was obtained in the 100 μm microchannel width with 10 mm hydrophobic patch. Therefore, it is noted that a 10 mm hydrophobic patch is the optimum size of the hydrophobic patch to obtain the maximum volume of the separated plasma in the blood plasma separator.

The width of the microchannel also affects the capillary force or moving tendency of the leading edges of the blood cells and the separated plasma depicted in Table 4.2. In the 50 μm width of the microchannel, strong capillary forces were induced in the leading edges of blood cells and separated plasma, so blood cells were not effectively retarded for accumulation in the region. As a result, a fairly small amount of blood plasma was eventually obtained from the whole blood shown in Figure 4.11(c). In the 200 and 400 μm width of the microchannel, both blood cells and the separated plasma were efficiently trapped within the patch region because of the relatively weak capillary forces in the leading edges. The movements of the components were rather slow and only a slight
amount of blood plasma was separated from the whole blood. A 15 nL volume of the separated plasma was also obtained in the 400 µm microchannel width of the separator, which is still not enough for the POCT, shown in Figure 4.11(c). Therefore, successful separation of the blood plasma from the whole blood was achieved in 100 µm microchannel with 10 mm hydrophobic patch because the blood cells were effectively retarded in the patch during the separation of the blood plasma.

<table>
<thead>
<tr>
<th>Microchannel width (µm)</th>
<th>Capillary force in the leading edge</th>
<th>Separated blood plasma (S1)</th>
<th>Blood cells (S2)</th>
<th>Side view of the separator (10 mm hydrophobic patch)</th>
<th>Top views of the separator (10 mm hydrophobic patch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Strong</td>
<td>Strong</td>
<td>Strong</td>
<td><img src="image1" alt="Side view" /></td>
<td><img src="image2" alt="Top views" /></td>
</tr>
<tr>
<td>100</td>
<td>Strong</td>
<td>Weak</td>
<td>Weak</td>
<td><img src="image3" alt="Side view" /></td>
<td><img src="image4" alt="Top views" /></td>
</tr>
<tr>
<td>200</td>
<td>Weak</td>
<td>Weak</td>
<td>Weak</td>
<td><img src="image5" alt="Side view" /></td>
<td><img src="image6" alt="Top views" /></td>
</tr>
<tr>
<td>400</td>
<td>Weak</td>
<td>Weak</td>
<td>Weak</td>
<td><img src="image7" alt="Side view" /></td>
<td><img src="image8" alt="Top views" /></td>
</tr>
</tbody>
</table>

**Table 4.2** Effects of the width of microchannel on the separation results of the blood plasma.
The percolation of red blood cells (RBC) at the separation patch in the microchannel is an important factor in the developed whole blood/plasma separator because this phenomenon is closely related to the blood plasma separation. In order to further explore the development of the percolation in the separation patch, the concentrations of whole blood were changed by serial dilution and the diluted bloods (3 \( \mu \)L) were tested through the separation channel. Therefore, the development of the percolation was observed with various concentrations with whole blood and 1X PBS buffer solution (diluent) based on the ratio to see if there was a threshold RBC’s concentration which was unable to achieve the separation. The separation images were captured in a 1.5 mm length out of a 10 mm hydrophobic patch because 1.5 mm was a region of interest (ROI) by microscope in this experiment. In order to determine the threshold concentration, the lengths of the blood cells were measured when the leading edge of the plasma reached 1.4 mm in length in the patch shown in Figure 4.12. A 10X dilution (1:9 = whole blood : 1X PBS buffer solution) was first performed to measure the threshold concentration. Figure 4.12 shows that blood plasma separation occurred in a 10x dilution. So, more serial dilutions such as 20X, 30X, 40X, 50X, 60X, and 70X dilutions were tried to track the percolation of RBC at the separation patch in the microchannel. The separation images of the plasma were obtained from no dilution to 40X dilution in Figure 4.12. In these dilutions (0 ~ 40X), the leading edges of the separated plasma were moving to the end of the patch (10 mm) even if the leading edges of the blood cells were still following those of the plasma. However, from the 50X dilution to 70X dilution in Figure 4.12, the blood plasma and the blood cells were
simultaneously moving to the 1.4 mm length, and finally reached the end of the hydrophobic patch region.

**Figure 4.12** Captured images to determine the threshold concentration for percolation of RBC's across the microchannel.
Based on these images, lengths of the blood cells in hydrophobic patch region were measured in terms of the whole blood dilution with PBS solution shown in Figure 4.13. It is noted that a threshold RBC’s concentration for the device developed in this work, which doesn’t achieve the separation, was obtained in a 50X dilution. The effective percolation of RBC in this separator is obtained in undiluted whole blood based on the experimental results. Therefore, the whole blood/plasma separator developed in our lab is very desirable for separating high quality of blood plasma from undiluted whole blood. This separator is considered one of the promising platforms for POCT using undiluted whole blood.

![Graph showing measurements of the length of blood cells in hydrophobic patch region in terms of the whole blood dilution with PBS solution. The threshold concentration was obtained in 50X dilution based on the Figure 4.12. The measurements were performed in five times.]

Figure 4.13 Measurements of the length of blood cells in hydrophobic patch region in terms of the whole blood dilution with PBS solution. The threshold concentration was obtained in 50X dilution based on the Figure 4.12. The measurements were performed in five times.
4.5 CONCLUSION

A new asymmetric capillary force driven on-chip whole blood/plasma separator has been proposed, fabricated and fully characterized for S-to-A microfluidic LOC platform in this work. Nanoporous superhydrophilic surfaces by spray LbL nano-assembly method and a patterned 10 mm hydrophobic patch were combined to attain asymmetric capillary forces through the microchannel for the separation of blood plasma from whole blood. A 50X dilution was a threshold RBC’s concentration, which didn’t achieve the separation, in the blood plasma separator developed in this work. Thus, we believe a stable percolation of RBC in this separator was formed at the undiluted whole blood. The blood plasma separator developed in this work has successfully separated high quality blood plasma from undiluted human whole blood without any external power source. This new separator is very suitable for the POCT platform because it is a simple, self-powered, tubing-free, and filter-free device. This separator with a simple asymmetric microchannel can be flexibly integrated into a ‘smart’ S-to-A microfluidic LOC devices for POCT applications.
CHAPTER 5

A WHOLE BLOOD SAMPLE-TO-ANSWER POLYMER LAB-ON-A-CHIP WITH SUPERHYDROPHILIC SURFACES
5.1 INTRODUCTION

As stated in Chapter 4, a blood test is the most common medical diagnostic tool to monitor the health condition of patients who have chronic diseases (such as diabetes) and is of great importance in global health applications. A blood test is also the best way in clinical diagnostic settings for urgent care patients in regions with poor resources, in the field, or in combat zones. This is because faster or more efficient treatment can be delivered due to the rapid and precise diagnosis of diseases with blood that contain critical information concerning the function of the whole body [1].

Detection of analytes in whole blood is challenging because of the complexity of the mixed constituents. Furthermore, separation of plasma from whole blood is a critical step for most bioassay-based clinical blood analysis to minimize the noise from blood cells as this interferes with the optical path. Through the impact of microfluidic LOC technology in various fields [12–14] a miniaturization of blood analytical systems can be realized, leading to shorter turnaround time, reduced sample consumption, lower cost, and better reproducibility in many applications. It is required to integrate the miniaturized on-chip blood plasma separator developed in Chapter 4 into the analytical system for the blood test. From the patient’s point of view, it is very desirable to design and develop a more practical blood analytical system. This system easily integrates an on-chip blood plasma separator with bioassay-based detection element that does not need an external power source. This device concept is a S-to-A polymer LOC platform, which means that results are obtained after the injection of a sample onto the platform with no further processing.
In order to realize this S-to-A polymer LOC platform for blood testing, the bioassay-based detection element must be assembled and integrated with the on-chip blood plasma separator developed in Chapter 4. Among various detection methods [105–119], a colorimetric assay [120–123] was selected as a demonstration vehicle for the capillary-driven lateral flow assay platform because the assays give visual readouts and are usually simple, stable, and low-cost compared with other assays. Furthermore, the colorimetric assay is also used in many diagnostic assays performed on blood plasma such as protein (e.g. bovine serum albumin), heparin, glucose, lactate, lysophosphatidic acid, and cholesterol [124–127]. Recently, many groups have implemented the standard colorimetric assays in paper-based microfluidic devices [128–131] because the technology for paper-based microfluidics offers many advantages for POCT. Fabrication of a paper-based microfluidic device is relatively simple and requires only a fraction of the time, resources, and infrastructure required for producing traditional diagnostic devices. The compact, low-cost, and simple to use platform for quantitative colorimetric assays is well-suited for implementation in the developing world and other poor-resource settings [132–139].

There are several good examples of paper-based colorimetric assay platforms. Whitesides’ group at Harvard University demonstrated a paper-based microfluidic device with colorimetric assay on chromatography paper patterned with photoresist [132] (Figure 5.1). The colorimetric assays for glucose and protein are successfully demonstrated for varying concentrations of glucose and BSA. His group also showed how to quantify colorimetric assays and to exchange the results of the assays digitally with physicians located off-site [133] (Figure 5.2). They have used paper-based
microfluidic devices for simultaneous multiple assays and camera phones for digitizing the intensity of color in the colorimetric assay results. They then transferred the digital information to an off-site laboratory for analysis by a trained medical expert with appropriate communication infrastructure; the results then can be returned directly to the provider in the field.

**Figure 5.1** An example of the colorimetric assay results on chromatography paper. The colorimetric assays for glucose and protein are demonstrated using various concentrations of glucose and BSA. Images adapted from [132].
Figure 5.2 An example of a colorimetric assay and the results of a digitized intensity diagram for both grayscale and cyan channels; the display engine is Adobe Photoshop. This result is then communicated to physicians located off-site. This figure shows the procedure for quantifying the levels of glucose and protein in urine. The concentration of the analyte can be obtained from the mean pixel values within the test zones. Images adapted from [133].
Henry’s group at Colorado State University used multiple indicators for a single analyte in paper-based microfluidic devices to improve the ability to visually discriminate between analyte concentrations [138] (Figure 5.3). Culbertson’s group at Kansas State University developed paper-based microfluidic devices using a polymer blend for monitoring several analytes such as urinary ketones, glucose, and salivary nitrite [139] (Figure 5.4).

**Figure 5.3** An example of colorimetric assays on paper-based microfluidic devices for the simultaneous semi-quantitative analysis of glucose, lactate, and uric acid. Images adapted from [138].
Figure 5.4 An example of a paper-based microfluidic chip with the colorimetric detection of salivary nitrite. Color intensities were quantified using a flatbed scanner and image manipulation software and plotted against concentration to produce calibration curves for the assay. Images adapted from [139].

A capillary-driven lateral flow assay platform is the simplest and best for commercialized POCT because it is portable, inexpensive, and only requires small sample volumes. In this type of assay, antigens can be detected in above certain thresholds because the lateral flow method excluded the requirement for the incubation and washing steps in a classic protocol. This lateral flow also increased the total number of captured analytes in a given time. A colorimetric assay is one of the main applications of the capillary-driven lateral flow platform. Many groups are now focusing on the capillary-driven colorimetric assay in paper-based microfluidic devices [128–131]. The detection principle for the colorimetric assay is usually the color-change reaction. Processing these colorimetric results to derive a binary yes/no answer or a semi-
quantitative detection is enough for primary diagnosis. The results of the reactions can also be visually analyzed by the unaided human eye with a simple color-matching chart. Thus, it is noted that this detection method is very desirable for physicians, nurses, and patients in the developing world, in the field, and in home-care settings. In spite of the many advantages of the capillary-driven lateral flow colorimetric assay using paper-based microfluidic devices, there are several limitations. These limitations include the material properties of paper, the fabrication methods of the device, and detection issues related to the devices. Specifically, the target analytes or proteins in the sample blood could be easily bound over the paper strip where the plasma should be separated from the sample blood. The unwanted and nonspecific binding of the molecules on the paper strip, which work like a filter for the blood/plasma separation, usually produce the low limit of detection (LoD) or the low coefficient of variation (CV). Due to these limitations, there is a large demand for the design and development of more practical blood analytical systems that easily integrate an on-chip blood plasma separator with lateral flow colorimetric assay-based detection components in an automated manner.

In this work, we propose a whole blood S-to-A polymer LOC platform that simply integrates an on-chip blood plasma separator with a capillary-driven lateral flow colorimetric assay component without an external power source as shown in Figure 5.5. The superhydrophilic surface (a key technology developed in Chapter 2) modified by a spray LbL nano-assembly method was designed for blood transportation in devices by capillary pumping, on-chip whole blood/plasma separation with an asymmetric capillary force, and detection using a capillary-driven lateral flow colorimetric assay. The whole blood S-to-A polymer LOC can be a good platform for the POCT because it is simple,
disposable, functional, and user-friendly platform. This platform is also favorable for mass production and commercialization because the thermoplastic COC used for the device fabrication is a good candidate for mass production by injection molding or hot embossing processes.

**Figure 5.5** Working principle of a S-to-A polymer LOC device with capillary-driven lateral flow colorimetric assay platform: (a) chip fabrication with spray LbL nano-assembly; (b) spotting reagent for colorimetric assay and whole blood injection; (c) on-chip blood plasma separation; and (d) color change due to the lateral flow colorimetric assay.
5.2 DESIGN AND FABRICATION

5.2.1 Microfabrication of S-to-A Polymer LOC Platform

Figure 5.6 Summary of fabrication process of the S-to-A polymer LOC device: (a) fabrication procedures; (b) spray LbL nano-assembly method; (c) device design; and (d) fabricated device.
Figure 5.6(a) summarizes the fabrication process of the S-to-A polymer LOC platform integrated with an on-chip blood plasma separator developed in Chapter 4. The procedure is similar to that developed in Chapter 4. A CNC milling machine was used for fabricating a master mold. The CNC milling machine manufactures various sizes of structures in the range of µm to mm in scale. The microchannel was designed to transport the introduced whole blood, measure the separated volume of plasma from whole blood, and to characterize the asymmetric capillary movement of blood plasma for colorimetric assay. The volume of the inlet reservoir was approximately 3 µL, which is comparable to the volume of whole blood taken from a finger prick using a commercial device. A microchannel in the dimension of 50, 100, 200, 400 µm width \( \times \) 100 µm depth \( \times \) 25 mm length and a spray coated surface with a 10 mm hydrophobic patch was designed for a S-to-A polymer LOC platform.

The master mold for the replica of COC microchannels as shown in Chapter 4 containing straight microchannels was fabricated on a 6061 aluminum alloy which was 4 inches wide, 4 inches long, and 0.25 inches thick. Then, COC substrates were patterned with microfluidic channels using a hot embossing technique for the spray LbL coating process. After this, the spray LbL nano-assembly process with silica nanoparticles [44] was performed for the asymmetric superhydrophilic surface with a 10 mm hydrophobic patch over the COC microchannel. Finally, the COC substrate with microchannels was bonded with the spray functionalized COC substrate by a thermoplastic fusion bonding technique using an embossing machine [75]. The designed microchannel with 400 µm \( \times \) 100 µm \( \times \) 50 mm (W \( \times \) D \( \times \) L) and the 10 mm hydrophobic patch are shown in Figure 5.6(c). The fabricated S-to-A polymer LOC device is shown in Figure 5.6(d). The
multilayer build-up process for the superhydrophilic surface by spray LbL is schematically described in Figure 5.6(b). A COC was prepared to fabricate the S-to-A polymer LOC platform. The COC surface, activated by oxygen plasma, was modified by successively spraying PDDA and PSS, diluted to 0.01 M in DI water, as an adhesion promoter. A rinsing step with DI water was performed after a complete layer was formed. Then, a layer of oppositely charged polyions and a rinsing solution were applied by enforced spraying as shown in Figure 5.6(a). 12 nm in diameter Ludox HS-40 colloidal silica nanoparticles, which are negatively charged and diluted to 0.3 wt % in DI water, were used for the spray LbL nano-assembly. The PDDA and silica nanoparticles were applied to the COC surface by spraying. Spraying was then suspended but drainage and evaporation of water continue until the prepared surface is completely dry. The deposited film was also spray-rinsed with DI water.
5.2.2 Preparation of Colorimetric Assay for Protein Quantification

The 0.3 μL of 500 mM citrate buffer solution (pH 2.5, Fisher Scientific, IL) was placed onto the superhydrophilic COC surfaces, followed by the layered 0.3 μL of 3.3 mM of tetrabromophenol blue (TBPB, Sigma-Aldrich, St. Louis, MO) in 95% ethanol (Fisher Scientific, IL) shown in Figure 5.7(b). The reagents on the superhydrophilic surface were allowed to dry at 25°C for 10 min.

**Figure 5.7** Preparation of colorimetric assay for protein quantification in a S-to-A polymer LOC platform: (a) a S-to-A polymer LOC device; (b) spotting the citrate buffer solution followed by TBPB in ethanol for colorimetric assay and drying for 10 min; (c) injection of the spiked whole blood into the device; and (d) color change due to the colorimetric protein assay.
Introducing the spiked whole blood into the S-to-A polymer LOC device, the blood plasma successfully separated from the whole blood was transferred to the detection zone for colorimetric assay described in Figure 5.7(d). This colorimetric protein assay uses the nonspecific binding of TBPB to proteins. The reaction is through a combination of electrostatic (sulfonate) and hydrophobic (biaryl quinone methide) interactions [141, 142]. When reacted, the phenol in TBPB deprotonates and the color of the dye shifts from yellow to blue, which indicates the presence of proteins in separated blood plasma [140, 142]. This colorimetric protein assay was calibrated and characterized with spiked concentrations ranging from ranging from 0 mg/mL to 5 mg/mL (0 μM to 75 μM) of BSA, which is clinically relevant range [132].
5.3 RESULTS AND DISCUSSION

5.3.1 Capillary-driven Lateral Flow Colorimetric Assay

Figure 5.8 shows a ‘smart’ S-to-A polymer LOC platform that integrates an on-chip blood plasma separator with capillary-driven lateral flow colorimetric assay. The reagents for colorimetric assay were placed onto the patterned superhydrophilic surfaces, which were modified by the spray LbL nano-assembly depicted in Figure 5.8(a). Single droplets of 3.0 µL human whole blood, a suitable amount for disposable single-use platform for POCT, spiked with different concentrations of BSA solution were injected into the inlets of the S-to-A LOC device shown in Figure 5.8(b). When the mixture of whole blood and BSA solution was injected into the inlet of the device, the top superhydrophilic surface generated capillary flow of whole blood through the microchannel. When the mixture encountered the 10 mm hydrophobic patch region, the flow of the whole blood was effectively retarded for a short period of time (< 1 min). This retarding effect caused a continuous selective accumulation of blood cells within the hydrophobic patch region. The movement of the blood cells were effectively reduced, allowing the blood plasma to move forward due to the differences in flow velocity between the cellular component and the blood plasma over the patch region. Thus, the blood plasma was successfully separated from the whole blood throughout this accumulated column of blood cells, a so-called ‘self-built-in blood cell microfilter’. The color changes from yellow to blue were obtained in Figure 5.8(b). The intensity of the blue color was increased depending on the concentrations of protein spiked in whole blood. Figure 5.8(c) shows a magnified image of moving separated blood plasma. This
picture demonstrates the filtering efficiency by asymmetric capillary force and the patterned hydrophobic patch.

**Figure 5.8** Capillary-driven lateral flow colorimetric assay in a S-to-A polymer LOC device: (a) spotting the layered 0.3 μL of 3.3 mM of TBPB in 95% ethanol over the 0.3 μL of 500 mM citrate buffer solution (pH 2.5) onto the superhydrophilic COC surfaces; (b) protein detection assays by using various concentrations of BSA; and (c) magnified view of the separated blood plasma in a 400 μm width microchannel.
**Figure 5.9** Volume of the separated plasma (nL) over time in the S-to-A polymer LOC platform; 400 μm width microchannel with a 10 mm hydrophobic patch. The volume of the separated plasma below 300 seconds was slightly increased over time due to the accumulation of blood cells over the hydrophobic patch during the separation. The volume of the separated plasma was rapidly increased after 300 seconds due to strong asymmetric capillary force throughout the ‘self-built-in blood cell microfilter’ developed in a 10 mm hydrophobic patch.
The movement of the separated plasma was monitored with an optical microscope by using reference marks along the microchannel. The volume of separated plasma was obtained by measuring the length of the leading edges between separated blood plasma and blood cells over time. The blood plasma shown in Figure 5.8(c) was successfully separated through a 5.8 mm long microchannel, where its cross-sectional area was 400 \( \mu m \times 100 \mu m \) (W \( \times \) D). A volume of 232 nL of separated plasma from a single drop (3 \( \mu L \)) of whole blood was finally obtained in a microchannel as shown in Figure 5.9. The summated total volume of the separated plasma from three microchannels in the device was used for blood analysis using capillary-driven lateral flow colorimetric assay. The summated volume of the separated plasma was large enough for on-chip POCT with undiluted human whole blood. The volume of the separated plasma below 300 seconds in Figure 5.9 was slightly increased over time due to the accumulation of blood cells over the hydrophobic patch during the separation. The volume of the separated plasma was dramatically increased after 300 seconds due to strong asymmetric capillary force throughout the ‘self-built-in blood cell microfilter’ developed in a 10 mm hydrophobic patch.

5.3.2 Calibration Curve for Colorimetric Assay with Spiked Proteins

The information about the concentration of protein in specific samples is required in the following three categories [143]:

(a) biological studies of protein expression;

(b) characterization and purification of proteins and enzymes;

(c) clinical diagnosis of transformed protein levels in body fluids
In clinical point of view, the quantification of proteins in human whole blood is significant in the diagnostics of various diseases. The variations in protein level in blood plasma or serum separated from whole blood is attributed from a variety of diseases such as blood disorders, digestive disorders, kidney disorders, and cancers [143].

In this work, the concentration of proteins spiked in whole blood was successfully quantified by the S-to-A LOC device using capillary-driven lateral flow colorimetric assay for two specific aims:

(a) To obtain calibration curve of protein concentrations in blood plasma separated from whole blood samples injected into the S-to-A LOC device; it is indicative of certain abnormalities in functions of human body if the protein concentration of the plasma is altered;

(b) To demonstrate the performance of the S-to-A LOC platform compared with that of the paper-based microfluidic device or conventional method: lower limit of detection, small sample volume, equipment-free (e.g. spectrophotometer), efficiency of plasma separation from real whole blood samples (e.g. high quality separated plasma)

In this assay, TBPB ionizes and binds with proteins to cause a color change. A positive result is indicated by a color change from yellow to blue. The intensity of the blue color is proportional to the amount of the proteins spiked in blood plasma or whole blood. To generate the calibration curve from the colorimetric assay in the device, the same method used in paper-based colorimetric assay was used shown in Figure 5.2 [133]. The images of the color changes were captured using a digital camera and the intensity was obtained with Adobe Photoshop in a gray scale mode (Figure 5.10). The mean values of the
selected area through Adobe Photoshop software correlate with the concentration of the spiked proteins in Figure 5.10(c).

Figure 5.10 Procedure for quantifying protein levels in separated blood plasma through a S-to-A polymer LOC device using Adobe Photoshop software: (a) colorimetric assay by using various concentrations of BSA; (b) conversion of photographed digital images to grayscale mode; and (c) a mean value within the selected test zone.
The intensity of color change was quantified by a calibration curve in terms of the protein concentrations in Figure 5.11. The regression equation is $\text{Intensity} = 8.1348 \ln(x) + 54.405$ ($R^2 = 0.8958$), where $x$ is the concentration of protein (in mg/mL). The colorimetric assay was calibrated with blood plasma samples separated from three microchannels with known protein concentrations ranging from 0 mg/mL to 5 mg/mL (0 μM to 75 μM) of BSA. The limit of detection was 0.025 mg/mL (25 μg/mL), which is lower than that from paper-based microfluidic device or conventional method [130, 138]. As a result, both blood plasma separation and quantitative analysis in whole blood was simultaneously demonstrated in a S-to-A polymer LOC platform that integrates an on-chip blood plasma separator with capillary-driven colorimetric assay. The quantified protein levels in S-to-A polymer LOC device can be a good clinical indicator of certain abnormalities in functions of human body such as blood disorders, digestive disorders, kidney disorders, and cancers. This platform is particularly useful in in poor-resource settings because of the small sample volume, equipment-free, high efficiency of plasma separation from real whole blood samples, and comparable limit of detection compared with the conventional methods.
Figure 5.11 Quantification of protein concentration in human whole blood samples using a S-to-A polymer LOC device with integrated on-chip blood plasma separator. The colorimetric assay was calibrated with blood plasma samples separated from three microchannels with known protein concentrations ranging from 0 mg/mL to 5 mg/mL (0 μM to 75 μM) of BSA. The limit of detection was 0.025 mg/mL (25μg/mL).
5.4 CONCLUSION

In this work, the ‘smart’ whole blood S-to-A polymer LOC platform was successfully developed integrating an on-chip blood plasma separator with capillary-driven lateral flow colorimetric assay component without any external power source. The superhydrophilic surface, which is a key technology developed in Chapter 2, modified by a spray LbL nano-assembly method was designed for three specific goals:

(a) lateral transportation of whole blood in the device by capillary pumping;
(b) on-chip whole blood/plasma separation with an asymmetric capillary force;
(c) detection using a capillary-driven lateral flow colorimetric assay

Clinically, the quantified protein levels in developed S-to-A polymer LOC device can be a good signature of various diseases in human body such as blood disorders, digestive disorders, kidney disorders, and cancers. The developed whole blood S-to-A polymer LOC device is an ideal platform for the POCT, particularly useful in poor-resource settings because it is simple, high-throughput, portable, low-cost, tubing-free, disposable, small sample volume, equipment-free, high efficiency of plasma separation, and a one-step diagnostic device. This platform is also favorable for mass production and commercialization because the thermoplastic COC used for the device fabrication is a good candidate for mass production by injection molding or hot embossing processes.
CHAPTER 6

CONCLUSIONS
6.1 SUMMARY

In this dissertation work, an innovative ‘smart’ S-to-A polymer LOC platform with superhydrophilic surfaces has been proposed, developed, and successfully characterized for POCT applications. The superhydrophilic surface (a key technology developed in this research) was fabricated by a spray LbL nano-assembly method. This product was designed for three specific goals: (a) lateral transportation of whole blood in the device by capillary pumping; (b) on-chip whole blood/plasma separation with an asymmetric capillary force; and (c) detection using a capillary-driven lateral flow colorimetric assay. Therefore, the developed S-to-A polymer LOC device, which integrates a new on-chip whole blood/plasma separator with a capillary-driven lateral flow colorimetric assay component without any external power source, is a generic platform for the POCT, particularly useful in poor-resource settings because it is a simple, high-throughput, portable, low-cost, tubing-free, disposable, and one-step diagnostic device.

A spray LbL nano-assembly method combining the LbL method and the spraying technique has been developed for preparing nanocapillary-driven superhydrophilic multilayer networks. This uses with 12 nm silica nanoparticles that are sprayed onto COC polymer surfaces. This modified assembly method is a powerful yet facile, practical, low-cost, easy-to-use (non-trained personnel), and high-throughput for selectively constructing nanoporous multilayer formation at room temperature. The major advantage of this method is a dramatically reduced processing time needed for the multilayer formation at room temperature. Surface functionality can be easily controlled by choosing appropriate polyelectrolytes. The resulting multilayer networks have been
successfully characterized by several methods. The dramatically dropped contact angles indicate that the COC surface should be superhydrophilic with silica nanoparticles due to the intrinsically high level of wettability of the silica nanoparticles coupled with the capillary imbibition of the LbL assembled nanoporous architectures. AFM measurement showed the surface roughness value was increasing proportionately with the number of silica bilayers assembled by spray LbL nano-assembly method. The superhydrophilic coating by spray LbL nano-assembly method was chemically stable over time.

A passive on-chip capillary pump with nanoporous superhydrophilic multilayer silica nanoparticle networks has been developed onto a hydrophobic COC microchannel using a spray LbL electrostatic nano-assembly method. The resulting multilayer networks on COC microchannels have been successfully characterized by two different methods and two testing platforms. The result confirmed that the on-chip capillary micropump drives a food dye in 47 mm straight microchannels without an additional power within 12 seconds. The capillary pumping effect decreased with increasing width of the microchannels. The pumping capability of the on-chip micropump is achieved from the strong hydrophilic properties of the nanoporous multi-coated bilayers of sprayed silica nanoparticles. The same platform with closed microchannels for on-chip blood plasma separation consistently separated about a 10 nL volume of blood plasma from whole blood. This separation result was mainly due to the strong asymmetric capillary force developed between a plain hydrophobic COC surface and the three nanoporous superhydrophilic surfaces in spite of the closed microchannels in the platform. These properties were mainly due to the nanoporous infiltration multilayer structures onto the COC microchannels.
A new on-chip whole blood/plasma separator driven by asymmetric capillary forces, which are produced through a microchannel with sprayed nanobead multilayers, has been designed, fabricated and fully characterized. Nanoporous superhydrophilic surfaces by a spray LbL nano-assembly method and a patterned 10 mm hydrophobic patch were combined to attain asymmetric capillary forces through the microchannel for the effective separation of blood plasma from whole blood without any external power resources. Blood cells were continuously accumulated over the hydrophobic patch while the blood plasma was able to flow over the patch. Then, the blood plasma was successfully separated from the whole blood throughout the accumulated blood cells which worked as a so-called ‘self-built-in blood cell microfilter’. The separated plasma was approximately 102 nL from a single drop (3 μL) of undiluted human whole blood within 10 minutes, which is suitable for single-use disposable POCT devices. This small amount of whole blood is important because separating an effective amount of blood plasma from a small amount of whole blood is very useful for neonates, pediatrics, resource-limited environments, and home-care settings for chronic disease. This separator with a simple asymmetric microchannel can be flexibly integrated into a ‘smart’ S-to-A microfluidic LOC device for POCT applications.

Finally, the concentration of proteins spiked in whole blood was successfully quantified with the ‘smart’ S-to-A polymer LOC device which was developed based on a capillary-driven lateral flow colorimetric assay. The intensity of color change was quantified by a calibration curve in terms of the protein concentrations ranging from 0 mg/mL to 5 mg/mL (0 μM to 75 μM) in blood plasma or whole blood. It is indicative of certain abnormalities in functions of the human body if the protein concentration of the
separated plasma is changed from known baselines. The limit of detection of the developed device was 0.025 mg/mL (25μg/mL), which is lower than that of paper-based microfluidic devices or conventional methods. As a result, both a high-quality of blood plasma separation and a quantitative analysis of whole blood were simultaneously demonstrated in a S-to-A polymer LOC platform that integrates an on-chip blood plasma separator with capillary-driven colorimetric assay. This platform is also suitable for mass production and commercialization because the thermoplastic COC used for the device fabrication is a good candidate for mass production by injection molding or hot embossing processes.
6.2 RESEARCH CONTRIBUTIONS

The major contribution of this research is to develop and demonstrate a new asymmetric capillary force driven on-chip whole blood/plasma separator, which is flexibly integrated into a ‘smart’ S-to-A polymer LOC platform for POCT applications. To the best of my knowledge, this is the first attempt to efficiently separate blood plasma from undiluted human whole blood using asymmetric capillary forces induced between hydrophobic and nanoporous superhydrophilic surfaces.

In order to achieve the asymmetric capillary forces between the surfaces, there is a critical need to effectively design the superhydrophilic surface, which is a key technology developed in this research, onto the naturally hydrophobic polymer substrates. A spray LbL nano-assembly method developed in this research allows preparing nanocapillary-driven superhydrophilic multilayer networks with silica nanoparticles onto the COC polymer surfaces. The lateral transportation of whole blood in the device is feasible by a passive on-chip capillary pump with sprayed multilayer networks. The pumping capability is mainly due to the nanoporous infiltration multilayer structures onto the COC microchannels.

Another innovative contribution of this research was to design and demonstrate a capillary-driven lateral flow colorimetric assay platform with superhydrophilic surfaces. The ‘paper-like’ superhydrophilic surfaces used in the S-to-A polymer LOC device enable us to overcome limitations in traditional capillary-driven colorimetric assay with paper-based microfluidic devices. It is noted that this detection method is very desirable for physicians, nurses, and patients in the developing world, in the field, and in home-care settings due to the simple visual read-out by the unaided human eye.
6.3 Suggestions for Future Work

An innovative ‘smart’ S-to-A polymer LOC platform with superhydrophilic surfaces has been successfully investigated for POCT applications in this dissertation work. However, there are several aspects of this technology suitable for future study.

Firstly, on-chip whole blood/plasma separation by asymmetric capillary forces used in this work can also be applied to other types of separation such as on-chip cell or particle separations for biomedical or biological applications.

Secondly, on-chip whole blood/plasma separation by asymmetric capillary forces used in this work can also be applied to more practical tube-type whole blood/plasma separation for clinical diagnostics. An innovative S-to-A lab-on-a-tube platform can be developed with a tube-type whole blood/plasma separator and detection elements integrated onto the flexible substrate.

Thirdly, the nanocapillary-driven superhydrophilic multilayer networks with silica nanoparticles by a spray LbL nano-assembly method can be deposited onto a variety of thermoplastic polymers that can be injection molded or embossed, such as PC or PMMA. A wide range of nanoparticles such as metal or magnetic nanoparticles can also be used for the spray LbL nano-assembly method onto the polymer surfaces to improve the sensitivity, specificity, and accuracy.

Finally, the detection limit in the capillary-driven lateral flow colorimetric assay can be dramatically lowered by spotting gold nanoparticles, carbon nanotubes or nano-wires onto the superhydrophilic detection zones. The ~ng/mL detection limit can be expected if the nano-scale materials above can be successfully utilized in the detection zone.
REFERENCES


PUBLICATIONS


