Janus-Type Wicking Microfluidic Devices for Separation and Collection of Plasma from Blood

by
Linus Unitan

A THESIS

submitted to
Oregon State University
Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Chemistry
(Honors Scholar)

Presented March 16, 2021
Commencement June 2021
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Linus Unitan for the degree of Honors Baccalaureate of Science in Chemistry presented on March 16, 2021. Title: Janus-Type Wicking Microfluidic Devices for Separation and Collection of Plasma from Blood.

Abstract approved:_____________________________________________________

Vincent Remcho

A simple Janus-type membrane-based wicking microfluidic device fabricated on a polycaprolactone-filled glass microfiber membrane enables separation and collection of blood plasma or serum from whole blood. This device bridges the wicking and cut-capillary-based solution transport mechanisms in a single membrane. The initial portion of the device is fabricated using selective oxygen radical exposure, a technique that enables accurate and precise construction of wicking channel geometries and their combinations. As the fresh blood continues to clot on the device, a separate fraction that is composed almost entirely of serum flows from the sample introduction zone down the channel. The other half of the device is fabricated with microscale capillaries cut into the hard-plastic portion of the membrane to guide and gather fluid into a collection zone. This facilitates blood plasma separation and collection in a single membrane at micro-scale sample and reagent volumes and at minimum cost. This device can be integrated with many diagnostic assays to develop inexpensive point-of-care diagnostic devices. It can also be used as a sample preparation tool to separate blood plasma or serum to aid with sample analysis performed outside of the device.

Key Words: blood separation, sample preparation, point of care diagnostics, plasma, serum, glass microfiber, polycaprolactone

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Introduction

Blood tests are essential for accurate diagnosis of diseases, and critical for ensuring proper patient care worldwide.\(^1,2\) The separation of blood plasma (the liquid fraction without clotting factors) or serum (the liquid fraction with clotting factors) from whole blood is one of the main requirements for accurate analysis, as the larger red blood cells interfere with some diagnostic tests. Colorimetric diagnostics, which rely on a change in color corresponding to the concentration of an analyte, are especially impacted by the red color interference from iron-rich hemoglobin in red blood cells.\(^3\) Traditionally, blood separation is accomplished in a laboratory environment through centrifugation by trained personnel, which makes blood diagnostics too expensive and impractical to deploy in resource-limited areas, such as rural communities, developing countries, and in emergency medical situations.\(^3\)–\(^5\) Effective separation of blood plasma or serum from whole blood at microliter scale and without the use of centrifugation is essential for efficient use of simple, inexpensive, and portable blood diagnostic and sample preparatory platforms.\(^6\)

Point-of-care wicking microfluidic devices are gaining popularity as an alternative to traditional laboratory analysis techniques.\(^7\) They are inexpensive and easily accessible, with existing devices (such as at-home blood glucose monitors) available over-the-counter.\(^8\) Point-of-care devices are easy to use with limited training, as pre-dosed reagents can be embedded within the device, and fluid flow is guided by the device itself.\(^9\) Microfluidic devices necessitate micro-scale sample and reagent volumes, which minimizes patient discomfort and limits reagent waste.\(^4\) Results from point-of-care microfluidic devices are available quickly, typically within a couple minutes.\(^3,7\)
Microfluidic devices often use capillary action to drive fluids without the use of pumps or other electricity-dependent components.\(^3\,^9\) Since the fluid transport mechanism of these devices is based on capillary action, it is challenging to separate the liquid phase from the device.\(^10\) Presently, this restricts the diagnostic capability of wicking microfluidic blood separators to assays that can be accomplished on the device itself, such as colorimetric analysis of an analyte in the separated plasma or serum fraction.\(^9\) Although the results of colorimetric diagnostics are convenient and easy to analyze with a hand-held colorimeter or by visual comparison to a reference chart, these tests have limitations in the wicking microfluidic format and are not suitable for every application. For example, color interference from the substrate matrix itself -- such as the near-white background of a glass microfiber membrane -- can lower the reliability of results in especially sensitive tests.\(^11\) Enabling separation and collection of blood plasma on a single device would expand the utility of point-of-care wicking microfluidic technology for use with any assay platform.

A simple Janus-type membrane-based wicking microfluidic device fabricated on a polycaprolactone (PCL)-filled glass microfiber (GMF) membrane was developed, which enables separation and collection of blood plasma or serum from whole blood. This device can be integrated as a front-end for many assays to yield inexpensive blood sample preparation. The device can be used with finger-prick volumes of raw blood, as well as blood treated with anticoagulants, enabling sample preparation at the point of care.
Materials and Device Fabrication Method

Figure 1 illustrates the Janus-type device fabrication steps described below. A completely biocompatible and biodegradable device with four separation and collection zones can be fabricated on a single membrane using this method at the manufacturing cost of roughly US $1.00 (~ US $0.25 per sample zone).

Chemicals

All solutions were prepared with deionized water from a Milli-Q purification system (Millipore Milli-Q Advantage A10) from EMD Millipore (Burlington, MA, USA) unless otherwise specified. Aqueous dye solution: red food dye from Kroger (Cincinnati, OH, USA). Blood surrogate solution: bovine serum albumin (BSA) from Sigma-Aldrich (St. Louis, MO, USA) with red food dye.

PCL-Filled GMF Membranes

Hydrophilic GMF membranes must first be made hydrophobic, which is accomplished by filling the GMF with PCL in two steps. PCL solutions (15 % w/v, 25,000 M.W) were prepared by dissolving appropriate weights of PCL (Perstorp, Warrington, UK) in appropriate volumes of analytical grade toluene (Macron Fine Chemicals, Center Valley, PA, USA). PCL solutions were spin-coated (Laurell WS-400, North Wales, PA, USA) at 2,500 rpm for 30 seconds on Whatman (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) glass microfiber (GF/A) membranes to ensure even application and saturation with PCL solution, followed by drying at 50°C for 15 minutes (Figure 1).
Once dry, half of each membrane was dipped into a thicker PCL/toluene solution (30% w/v, 40,000 M.W) until saturation was achieved, indicated by the submerged portion of the membrane turning translucent. These membranes were allowed to dry at room temperature overnight, then passed through a thermal laminator (TL902, 3M, St. Paul, Minnesota, USA) to ensure the hard plastic portion of the device had a smooth and uniform surface.

**Laser-Cut Elements**

Microscale capillary channels were cut with a laser cutter (VLS 3.50, Universal Laser Systems, Scottsdale, AZ, USA) into the hard plastic portion of the device under optimized settings. These capillaries guide fluid flow into a collection zone, which was rastered onto the device with a laser cutter.

The desired oxygen radical exposure mask for each surface of the membrane (top and bottom) was designed using drafting software (SolidWorks 2013-2014 Education edition, Waltham, MA, USA) and cut out of masking tape (i tape, Intertape Polymer Group, Montreal, CA) using a laser cutter with the following settings: power = 40% speed = 100%, pulses per inch = 500, z-axis = 0 (the thickness of a single layer of tape was assumed to be negligible) (Figure 2). The pattern contains a flow-through via to generate a 3D fluid flow channel geometry through the membrane, which enables efficient blood plasma separation. The downstream end of the plasma separator in the wicking fluidic section is connected with the laser cut features. The oxygen radical exposure mask can be designed to include additional features, such as multiple sample inlets and pre-treatment assay zones.
Oxygen Radical Exposure

Oxygen radical exposure (ORE) was accomplished using an Evactron (Redwood City, CA, USA) decontaminator/RF plasma cleaner installed on a home-built vacuum chamber. The pressure and forward RF power were maintained at constant values of 0.6 Torr and 13 W, respectively. Selective exposure to radicals was accomplished by covering the area of the membrane intended to remain hydrophobic and unexposed with a patterned mask, prepared as described above (Figure 2). The masked device was first placed face-up (the surface with sample and assay zone directed towards the oxygen radical source) into the homebuilt oxygen plasma exposure system and exposed to oxygen radicals for 8 seconds under the above vacuum conditions. Then the device was flipped into a face-down orientation (the surface with the bottom surface flow channel directed towards the oxygen radical source) and exposed to oxygen radicals for 4 seconds under the same conditions. The mask was removed to reveal the completed, ready-to-use device (Figure 3).
Figure 1. Summary of the fabrication approach for a Janus-type blood separation and collection device. Half of the PCL spin coated GMF is dipped into a viscous PCL solution to create a hard plastic portion onto which microscale capillaries are cut. The device is masked with tape and exposed to oxygen radicals in order to generate hydrophilic channels to guide fluid flow.
Figure 2. SolidWorks sketch of the wicking, cut-capillary, and collection well feature dimensions (mm). The "top mask" and "bottom mask" components of the sketch are cut out of tape with the laser cutter and applied to the top and bottom surfaces of the device for selective oxygen radical exposure. The "cut capillary channels and collection wells" are cut or rastered into the hard plastic portion of the device.

Figure 3. Diagram of a complete Janus-type wicking microfluidic device for blood plasma separation and collection with relevant features identified.
Methodology

Device Design, Optimization, and Testing with Aqueous Dye Solution

Devices were initially tested and optimized with aqueous dye solution to establish preliminary functionality, using the same initial membrane fabrication steps as outlined above. The laser-cut features of the device (cut-capillary channels and collection wells) were optimized as described in the following sections.

Cut-Capillary Channel Optimization

Capillary channels were fabricated using different laser cutter settings (Figure 4). The laser cutter settings of pulse-per-inch (PPI) for resolution, percent speed, and power were optimized using a high power density focusing optics lens (HPDFO – Universal Laser Systems, Scottsdale, AZ, USA). The laser cutter settings which generated deep and regular channels that did not cut all the way through the membrane were power = 10%, speed = 75%, pulses per inch = 1000, z-axis = 0. These settings were used in generating the capillary channels of the device. Laser cutters burn or vaporize material by directing a focused laser beam at the material's surface from the top-down, so the cut-capillary channels are wider at the top of the channel than at the base. The settings above generate regular features about 130 micrometers wide at the top of the channel and about 60 micrometers wide at the base of the channel. The collection wells were rastered into the device using the following settings: power = 50%, speed = 75%, pulses per inch = 500, z-axis = 0. The laser cutter settings listed above can be further optimized to change the capillary channel geometries (width, depth) for different substrate compositions or different application requirements.
Figure 4. (a) Maximum width (top of channel) and (b) minimum width (base of channel) of cut-capillary channels. These channels were cut with optimized laser cutter settings to generate even features without perforation of the PCL-filled GMF membrane to keep the channels intact. Aqueous dye solution was added to the channels to highlight channel boundaries.
Capillary and Collection Well Optimization

This fluidic channel arrangement was tested with aqueous dye solution. Decreased collection well size concentrates the fluid into a smaller surface area, aiding in yield and ease of fluid aspiration (Figure 5 a, b). Employing a 3D fluid flow wicking pattern ensures that all fluid that arrives in the collection wells has interacted with the device substrate (Figure 5 a), avoiding the possibility of fluid flow across the top surface of the device (Figure 5 b). Fluid collection yields are improved by increasing the number of capillary channels that feed a single collection well, as if one channel clogs or malfunctions, other channels are still capable of delivering fluid to the collection well (Figure 5 b). Up to 3 µL of aqueous sample volume is collected from each device with 3D fluid flow patterns out of an initial sample volume of 10 µL (Figure 5 a).

Channel length may also be changed to suit different applications. Cut-capillary channels feed the main collection well while also serving as collection zones themselves. Longer channels (Figure 5 a) provide more volume for fluid to congregate, enabling efficient fluid extraction from the device substrate. However, longer channels are more likely to clog or dry out, which may limit collection yield. Decreasing the length of the cut channels decreases the unusable wetted volume of fluid left behind on the device, but these devices require repeated aspiration, as less fluid is extracted from the ORE wicking portion at a time (Figure 5 c). Collection yields in devices with shorter channels are more consistent than in devices with longer channels. A yield of between 2 and 3 µL is standard and achievable in over 90% of testing zones (n=16), but the repeated aspiration necessary to obtain these volumes is more time consuming, averaging about 4.5 minutes for each zone.
Figure 5.
(a) Device with flow-through geometry tested with aqueous dye solution. Decreasing the size of the collection wells improves collection yields, as fluids are more concentrated and can be aspirated more effectively. Implementing 3D fluid flow architecture on the wicking side restricts fluid flow across the top surface of the device and ensures all fluid interacts with the device substrate.
(b) Device with flow-across geometry with varying numbers of cut channels per collection zone.
(c) Decreasing the length of the cut channels decreases the volume of fluid left behind on the device, further increasing collection yields.
Device Design, Optimization, and Testing with Blood Surrogate

As the main intended function of the device is in blood diagnostics, further optimizations were conducted with a 70 mg/dL solution of BSA to mimic the serum component of human blood.

Solution leakage within the membrane was evidence of reduced fluid flow control, and contributed to relatively poor collection yields. When membranes are left intact, less than 1.5 µL out of an initial sample volume of 10 µL was collected on average (n=16) (Figure 6 a). Isolation of fluidic features improves device performance, as extra void space is minimized when fluidic features are cut apart from the rest of the membrane (Figure 6 b). When the relevant features are cut out to limit extra available void space for the solution to leak into, the collection yields increase to around 2 µL out of an initial sample volume of 10 µL (n=8).

Figure 6. (a) Complete and (b) cut-apart devices tested with blood surrogate solution. Cutting out the relevant features limits extra available void spaces, decreasing fluid leakage into the membrane and improving fluid collection yields.
The molecular weight and thickness of PCL (used in both spin coating and dip coating) was further optimized to limit fluid leakage within the void spaces of the membrane (Figure 7 a, b). Membranes that were spin-coated with PCL solutions of higher thickness and molecular weights show decreased fluid leakage in the wicking portion of the device and increased pattern resolution. When fluid was applied onto an unexposed region of the device, it behaved as expected and remained a droplet on the surface that did not seep into the membrane.

Figure 7. GMF spin-coated with either (a) CAPA 6250 (25,000 MW) 15% w/v PCL in toluene, or (b) CAPA 6506 (50,000 MW) 15% w/v PCL in toluene. Both devices were tested with BSA blood surrogate.
Device Testing with EDTA Treated Canine Blood Samples

This Janus-type device was designed to expand the utility of a wicking microfluidic blood separation device developed by the Remcho group. A device was fabricated under conditions optimized with aqueous-dye solution and tested with healthy canine blood (Figure 8). This blood sample was treated with EDTA anticoagulant prior to introduction to prevent clogging of the separation channel and maximize collected plasma volume. About 1 µL of plasma was collected out of the 10 µL of blood introduced into the sample zone of the device. Since roughly half of mammalian blood is plasma by volume, this result represents a collection rate of about 20%.

![Figure 8. Functional section of a device used with EDTA-treated healthy canine blood samples](image)

Devices fabricated with parameters optimized with BSA-based blood surrogate were tested with EDTA-treated healthy canine blood (Figure 9 a) and compared to devices fabricated using settings optimized under previous work (Figure 9 b). Although the ORE fabricated wicking sections of the device appeared more well-defined under optimized conditions, resulting in
significantly improved fluid control, blood separation was either limited or unsuccessful.

Decreased loss of fluid into the void spaces of the surrounding substrate material was insufficient to overcome the decrease in blood separation capability, and plasma was not collected from devices fabricated with settings optimized for fluid control (n=8).

Figure 9.
(a) BSA-optimized fabrication method: CAPA 6506 (50,000 MW) 15% w/v PCL in toluene, (i) front side, (ii) back side
(b) Original fabrication method: CAPA 6250 (25,000 MW) 15% w/v PCL in toluene, (i) front side, (ii) back side
High Throughput Fluid Introduction and Collection

A pipette was primarily used to introduce solutions into the devices and aspirate solutions from the collection wells to closely represent the real-world point-of-care diagnostic applications of this device. However, this device can also be used in high throughput applications. For example, a syringe pump can exert a constant aspiration force and extract discrete droplets from the laser rastered collection well while additional sample is gradually introduced into the sample zone in the wicking section of the device (Figure 10 a). Fluid extracted by the syringe pump can be expelled and gradually collected on another medium. Simultaneous sample introduction and aspiration encourages fluid flow and prevents the channel from drying, clogging, and becoming unusable. Although the syringe pump can increase fluid yields, the efficacy of this method depends on using appropriate extraction rate and tubing material for a given sample composition and volume. Aqueous dye solution was successfully aspirated and expelled using the syringe pump (Figure 10 a), however, the surrogate protein solution droplets broke up within the tubing and were unable to be expelled as-is (Figure 10 b). The tubing could be rinsed with a known volume of water into another vessel, yielding a diluted solution.
Figure 10.
(a) A cut-apart device attached to a syringe pump. Aqueous dye solution was gradually introduced to the wicking channel by pipette while a constant aspiration force was applied to the laser-rastered collection well by the syringe pump.
(b) A second cut-apart device was tested with BSA-based blood surrogate solution. Due to the change in surface tension as a result of the solutes, the solution droplets did not stay intact, so they cannot be easily expelled by the syringe pump for collection.
Gated Fluid Control

Slight overlapping the wicking and cut-capillary features allows fluid to flow continuously between the two halves of the device, enabling fluid collection. Continuous fluid flow is prevented when wicking and cut-capillary features are slightly offset, introducing one-way gate functionality. Fluid introduced into the wicking portion of the device does not continue to flow into the cut capillaries, as its movement is restricted by the substrate matrix. In contrast, fluid introduced to the cut capillaries will reach the wicking portion of the device (Figure 11), as its movement through the cut-capillary channels is not as restricted. This functionality enables integration of separate multi-step reactions on a single wicking microfluidic platform.

Figure 11. Flow-across device illustrating gated fluid control. The red dye does not travel from the ORE wicking portion of the device to the cut-capillary channels, but the blue aqueous dye can travel from the cut-capillary channels to the ORE wicking portion of the device, displacing the red dye.
Further Applications

Examples of Potential Downstream Assay Chemistries and Detection Methods

The PCL-filled GMF substrates are chemically inert and compatible with many biological assays, and thus are ideal for use in point-of-care blood diagnostics. Janus-type wicking microfluidic devices enable collection of separated plasma, which allows the device to be used as a sample preparation process prior to many blood-based diagnostic wet chemistries that would be impractical to accomplish on the device itself (eg. enzyme-linked immunosorbent assay (ELISA)\textsuperscript{14}, Treponema pallidum hemagglutination assay\textsuperscript{15}) or based on proprietary technology, such as Vitros\textsuperscript{®} slides.\textsuperscript{16} Other assay chemistries, such as sample pre-treatment or end assays, can be incorporated into the device before or after the oxygen radical exposure step. This allows this technology to function as an inexpensive wicking microfluidic blood sample preparation device as well as a complete diagnostic device.
Conclusion

The Janus-type microfluidic device fabrication approach offers several advantages over existing blood separation methods. It uses low cost materials, such as glass microfiber membranes, PCL, and tape mask material. The fabrication method consists of steps that are easily automated, such as membrane spin-coating and oxygen radical exposure. On-device blood separation is achieved with minimum sample volume (~10 µL), minimizing patient discomfort and biohazard waste. With further optimization, these devices are capable of handling high throughput and high volume applications. These devices are compatible with raw blood and blood treated with anticoagulants. They are easy to use with little or no additional sample preparation, so they can be used with little training. These devices enable integration of on-device sample preparative and end assay chemistries using microscale reagent volumes, and are suitable for a broad range of detection methods, both on and off-membrane.
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