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Patterned polycaprolactone-filled glass microfiber microfluidic devices for total protein content analysis

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ABSTRACT

Membrane based microfluidic devices have gained much popularity in recent years, as they make possible rapid, inexpensive analytical techniques that can be applied to a wide variety of areas. The ability to modify device hydrophilicity and hydrophobicity is critically important in fabricating membrane based microfluidic devices. Polar hydrophilic membranes, such as glass microfiber (GMF) membranes, hold great potential as they are inexpensive, chemically inert, and stable. Filling of these membranes with non-polar polymers such as polycaprolactone (PCL) converts the hydrophilic GMF into a hydrophobic medium. Controlled alteration of the surface chemistry of PCL/GMF substrates allows for the fabrication of microfluidic patterns on the surface. Using this approach, we have developed a simple and rapid technique for fabrication of highly adaptable complex multidimensional (2D and 3D) microfluidic pathways on a single membrane. PCL-filled GMF media were masked and selectively exposed to oxygen radicals so that the exposed surface became permanently superhydrophilic in its behavior. The desired microfluidic pattern was cut into the mask prior to assembly and exposure, and the mask was removed after exposure to reveal the ready-to-use microfluidic device. To verify and demonstrate the performance of this novel fabrication method, a colorimetric total protein assay was applied to the determination of protein concentrations in real samples.

1. Introduction

Wicking microfluidic analytical devices have been applied to many areas of analytical chemistry [1–3]. These analytical devices offer rapid and reliable measurements at low cost. The quality of these devices and their range of applicability are highly dependent upon the method of fabrication applied and materials of manufacture [4–7]. The basic fabrication method for devices of this kind involves patterning of a support substrate (an organic or inorganic filtration membrane) into hydrophilic channels defined by hydrophobic barriers comprised of various polymers, water resistant inks/toners or waxes [5,8,9].

As a supporting substrate, glass microfiber (GMF) membrane media is a good candidate that offers many advantages over paper. GMF is an inexpensive, temperature stable, pH resistant, and chemically inert hydrophilic membrane made up of fine microscale borosilicate fibers [10,11]. This study introduces a combination of a GMF substrate with a polycaprolactone (PCL) barrier agent as a novel platform for the fabrication of wicking microfluidic devices. The GMF substrate can be adapted for use with many different chemistries and assays when used in concert with PCL, a biocompatible and biodegradable polymer that can be used to define the hydrophobic portions of these devices [12]. PCL is a polyester with low melting and glass transition temperatures, high miscibility with other polymers, excellent solvent compatibility, and a facility for functionalization [13,14]. Hence, it is favored for use in various applications that expand device applicability [13–15].

Cost and complexity are key factors of importance in designing microfluidic devices for broad application. Therefore, development of simple and inexpensive fabrication techniques that do not require expensive chemicals or instruments is a necessity. Oxygen plasmas have been employed as microfluidic device fabrication tools, primarily to affect surface chemistry, by many groups [16–19]. These are strong plasmas that rigorously etch surfaces. On the other hand, oxygen plasma decontaminators have been widely used for gentler cleaning processes for many years [20–22]. Decontaminators are optimized to generate long lived oxygen radicals rather than strong O_2 plasmas [23]. Exposure of a polymer-coated substrate to oxygen radicals will effectively alter surface properties, such as surface free energy, which are the driving forces that dictate the hydrophilic/hydrophobic nature of the surface [24,25].

In this study, a novel, low cost, simple, and highly-adaptable microfluidic device fabrication approach was developed for PCL-filled

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GMF membranes. This fabrication approach involves selective exposure of the PCL-filled GMF membranes to oxygen radicals which is achieved using a mask made from an inexpensive tape. Changes in the mask design allow for fabrication of different channel geometries (flow-through, flow-through + lateral flow, and surface-lateral flow). Combining such different channel geometries allows for fabrication of complex multidimensional (2D and 3D) microfluidic devices on a single polymer-filled membrane, enabling unique properties and applications. Traditional approaches for fabricating 3D flow devices typically involve multistep stacking of 2D patterned layers/membranes using intermediate layers with adhesive properties [26,27]. Thus, these devices require multiple layers of materials and multiple unit operations to fabricate. In contrast, by using the selective oxygen radical exposure fabrication technique described here, microfluidic mixers, separations tools, delay circuits, and timing devices can all be fabricated on a single membrane that contains all needed 3D fluid flow pathways. Surface flow (2D) assay immobilization zones are highly useful in performing colorimetric assays. This approach allows for assay components to be immobilized on the surface the membrane and generates an increased analytical signal as it is largely free from the membrane matrix effects. This fabrication approach also allows for deposition of assay components before and/or after the fabrication of fluidic channels, as best accommodates the assay reagent requirements. In this study, we have demonstrated the compatibility and performance of the fabrication technique by analyzing real world protein unknowns using an immobilized colorimetric assay for total protein quantitation.

2. Materials and methods

2.1. PCL-filled GMF membranes

PCL solutions (w/v) 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). Solutions were spin-coated (Laurell WS-400, North Wales, PA, USA) at 2500 rpm for 30 s on Whatman (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) glass microfiber (GF/A) membranes to ensure even application and penetration, followed by drying at 50 °C for 15 min. To date, the most impressive results have been achieved using PCL of 25000 M.W. at 15% w/v (in toluene), where the initial weight percentage of PCL: GMF was approximately 50:50 under the above conditions.

2.2. Preparation of masks

The desired mask for each surface (top and bottom) was designed using drafting software (SolidWorks 2013–2014 Education edition, Waltham, MA, USA) and cut out of tape (i tape, Intertape Polymer Group, Marysville, MI, USA) using a laser cutter (VLS 3.50, Universal Laser Systems, Scottsdale, AZ, USA) with the following settings: power = 40% speed = 100%, pulses per inch = 500, z-axis = 0.

2.3. Oxygen radical exposure (ORE) experiments

Oxygen radical exposure experiments were conducted using an Evactron (Redwood City, CA, USA) decontaminator/RF plasma cleaner installed on a home built vacuum chamber under conditions determined separately for each experiment. The pressure and forward RF power were maintained at constant values of 0.6 Torr and 13 W, respectively. Selective exposure to radicals – not to the plasma but only to radicals generated by the plasma – was accomplished by covering the area of the membrane intended to remain unexposed with a patterned mask, prepared as described above, and ensuring that the substrate was placed at a distance well below the plasma region. Exposure time was dependent on pattern requirements. The minimum demonstrated exposure time required to generate a microfluidic pattern was 5 s.

2.4. Protein analysis

Analysis of total protein content in various samples was conducted using an immobilized colorimetric assay consisting of a pH 1.8, 250 mM citrate buffer (95% ethanol: 5% water) and 3.75 mM tetra bromophenol blue (TBB) as a colorimetric indicator (Sigma-Aldrich, St. Louis, MO, USA), prepared in ethanol (Sigma-Aldrich, St. Louis, MO, USA). Assay reagents were immobilized as a 2:1 mixture of buffer solution: TBB solution using an automated solution dispenser (D300, HP Corvallis, OR) over the reagent zone (3 mm diameter) prior to

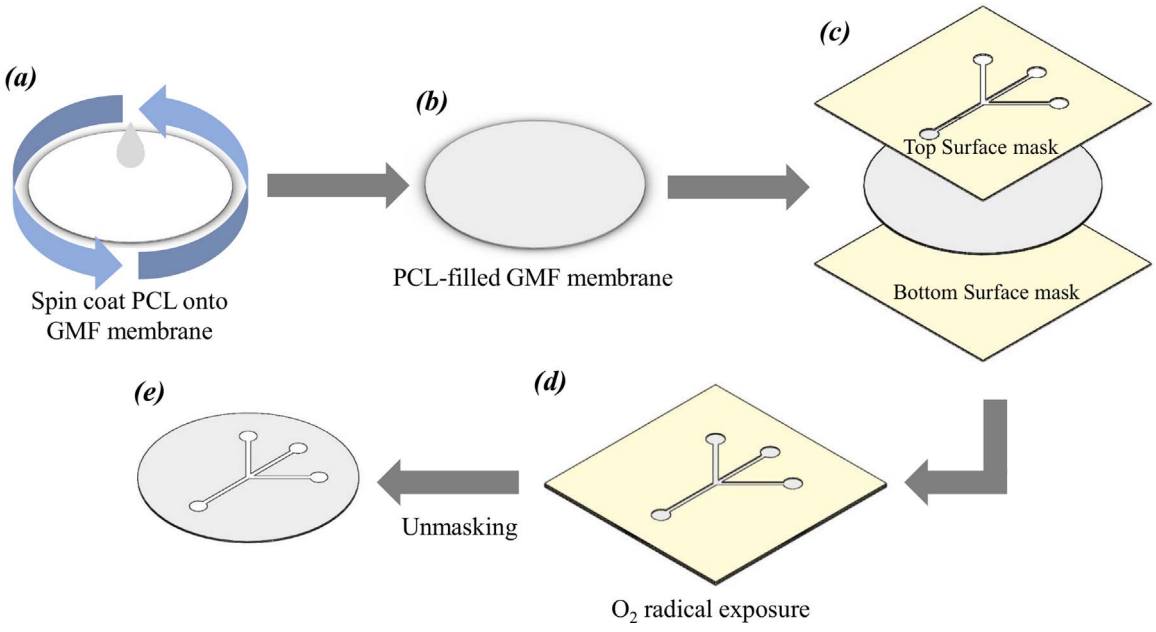

Fig. 1. Pattern fabrication mechanism: (a) PCL was spin-coated onto the GMF membrane to yield a PCL-filled GMF substrate (b). (c) The PCL filled GMF membrane was then sandwiched between the top mask (facing towards the oxygen radical source) and bottom mask (facing away from the oxygen radical source). (d) The membrane assembly was exposed to oxygen radicals. (e) Unmasking the substrate reveals the hydrophilic pattern on the PCL-filled GMF membrane.

fabrication of the fluidic channel by selective oxygen radical exposure. Triton X-100 (Fisher Scientific, Fair Lawn, NJ, USA) was added to the above immobilization mixture in order to ensure accurate dispensing (final surfactant concentration in immobilizing reagent mixture: 0.1%). Bovine serum albumin (Amresco, Solon, Ohio, USA) solutions prepared in pH 7.4 phosphate buffered saline were used as protein standards. Normal human serum (EMD Millipore, Temecula, CA, USA) was used as a protein unknown. Images of the developed assays (standards and unknowns) were collected using a digital single lens reflex (DSLR) camera (Nikon Inc. Melville, NY, USA) and a photo light box (Sanoto MK45, Amazon.com). The average RGB color space data for the assay zone was analyzed using image-j (National Institute of Health, USA) software [28].

3. Results and discussion

3.1. Fabrication of microfluidic devices

The ability to modify device hydrophilicity and hydrophobicity is of the utmost importance in fabricating wicking microfluidic devices. Filling of GMF membranes with non-polar polymers such as PCL endows the hydrophilic GMF with hydrophobic features. The spin coating approach to PCL filling results in a nano-scale PCL coating on the borosilicate fibers as shown in Fig. S1. This changes the average thickness of both the individual fibers (as shown in the SEM image in Fig. S1) and the thickness of the entire membrane (from 260 μm [29] to 292 μm (n = 40)). Subsequent controlled alteration of the surface chemistry of the PCL/GMF substrates allows for the fabrication of hydrophilic microfluidic patterns.

Fig. 1 illustrates the device fabrication process. The first step involves the conversion of hydrophilic GMF membranes to hydrophobic by filling them with PCL. Then the top and bottom surfaces of the PCL-filled GMF substrate were masked with separate pattern masks cut from inexpensive masking tape (Fig. 1c). The pattern was cut into the masks using a laser cutter, though it is possible to use other cutting tools as well. The substrate was then exposed to oxygen radicals (Fig. 1d) in the homebuilt exposure chamber. The patterned mask selectively exposed the PCL-filled GMF membranes to oxygen radicals, which altered the hydrophobic substrate-polymer surface chemistry to generate hydrophilic fluid flow pathways. The tape masks were then removed, revealing the hydrophilic patterns on the PCL filled membrane (Fig. 1e). The PCL filler renders the GMF membrane more rigid and monolithic, which, together with the weakly adhesive nature of the tape on the smooth hydrophobic surface, allowed for facile removal of the mask without damage to the underlying membrane.

Good contact between the mask and the PCL/GMF substrate and optimization of the oxygen radical exposure conditions are necessary to prevent over-activation of the surface, which led to inaccurate patterning. The optimum power and exposure time for oxygen radical production are highly dependent on the requirements of the pattern. Higher power and longer exposure times facilitated one-step fabrication of flow-through devices, while shorter exposure times and low power conditions allowed for fabrication of single-surface flow patterns. The molecular weight and weight percentage of the polymer determined the extent of conversion of hydrophilic GMF membranes into hydrophobic substrates. Optimization of polymer molecular weight and weight percentage (Fig. S2) and application mechanism (e.g. spin coating, dip coating, etc.) are also important (Fig. S3) in that they dictated polymer density and uniformity. As shown in Fig. 2, accurate dimensions at the sub millimeter scale can be rendered under optimum conditions. The smallest channel dimension (width) obtained reproducibly in this study was 250 μm.

Covering one side with a pattern mask developed that particular pattern on the respective surface, dictating the extent of fluid flow only on that surface. Masking both surfaces with the same pattern mask and exposing both surfaces for equivalent exposure times resulted in development of the pattern through the membrane, such that the addition of solution onto one surface wetted the other side of the device as well. The depth of the hydrophilic channels was controlled by regulating the exposure time (Fig. S4 a). As oxygen radical interaction with the substrate converts the membrane from hydrophobic to superhydrophilic in nature [30], channels fabricated using this approach behave as superhydrophilic conduits; therefore, aqueous solutions will flow rapidly though these channels and the total volume required to saturate the channel is small and predictable. Flow-through channels produced using this approach, however, define comparatively higher volumes than surface-flow channels.

Proper masking along with limited and controlled exposure to oxygen radicals allows for fabrication of different patterns (surface-lateral flow) on each face of a single membrane, facilitating two solution flows without mixing as shown in Fig. 3a. These surface channels can be connected using flow-through *vias* to generate versatile, complex, and useful 3D fluid flow devices in a single membrane as shown in Fig. 3b and Fig. S4 b. The device in Fig. 3b, for example, generates differential flow from each of two separate fluid inlets such that a segmented combined flow results. Video S1, S2 and S3 in the Supplementary Material provide visual evidence of how these devices perform.

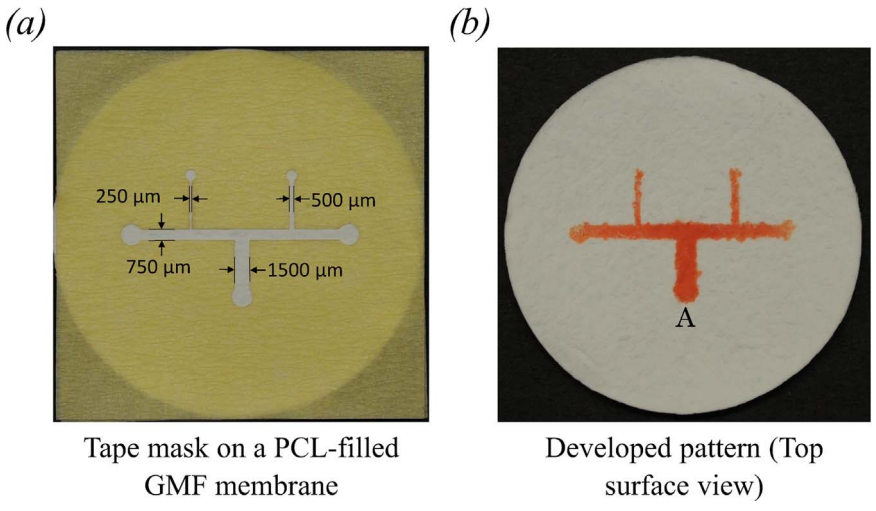

Fig. 2. (a) Fabrication of microfluidic channels having various dimensions (widths) using tape masks to define the required dimensions. (b) After oxygen radical exposure, the mask was removed to reveal the patterned membrane, the results of which are shown here by adding 8 μL of red food dye to spot "A".

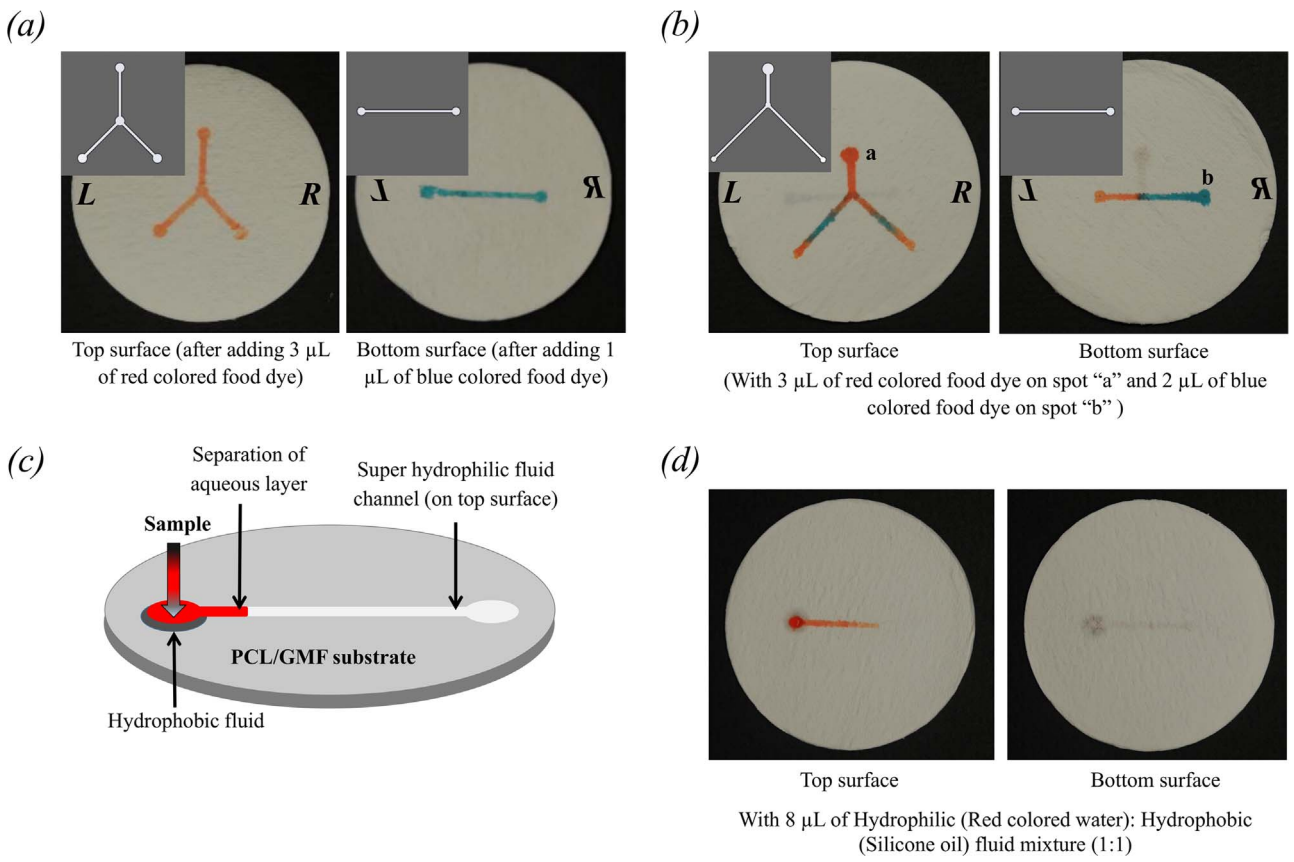

Fig. 3. Surface-lateral flow channel fabrication approach and applications. (a) Fabrication of separate surface-lateral flow patterns on each surface of a single membrane. (b) Surface channels can be connected using flow-through *vias* to generate differential flow from each of two separate fluid inlets such that a segmented combined flow results. (c) Microfluidic separation platform. (d) Separation of hydrophilic and hydrophobic fluids in an emulsion on the microfluidic separation platform. Upon the addition of the emulsion, the hydrophilic layer moved through the superhydrophilic surface channel and the hydrophobic layer was gradually absorbed into the PCL/GMF matrix. (The insert shows the respective mask design for each surface).

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The ability to use oxygen radicals to generate adjacent superhydrophilic and hydrophobic features on a single PCL-filled GMF membrane facilitated the production of an effective microfluidic phase separation platform (Fig. 3c). In the presence of an aqueous /organic emulsion fluid stream, the surface lateral-flow channel attracted the aqueous layer and transferred it rapidly through the channel while the hydrophobic fluid remained behind as shown in Fig. 3d. This simple and effective microfluidic device separated and transported aqueous layers from an emulsion (Fig. 3d). This basic approach to fabrication allows for many design and operational goals to be accomplished. These are discussed in more detail in the Supplementary Information provided online.

3.2. Demonstration of utility: a clinical assay for total protein content in a PCL filled GMF device

Devices produced using the approach detailed above were used to conduct a colorimetric total protein assay. The device was fabricated

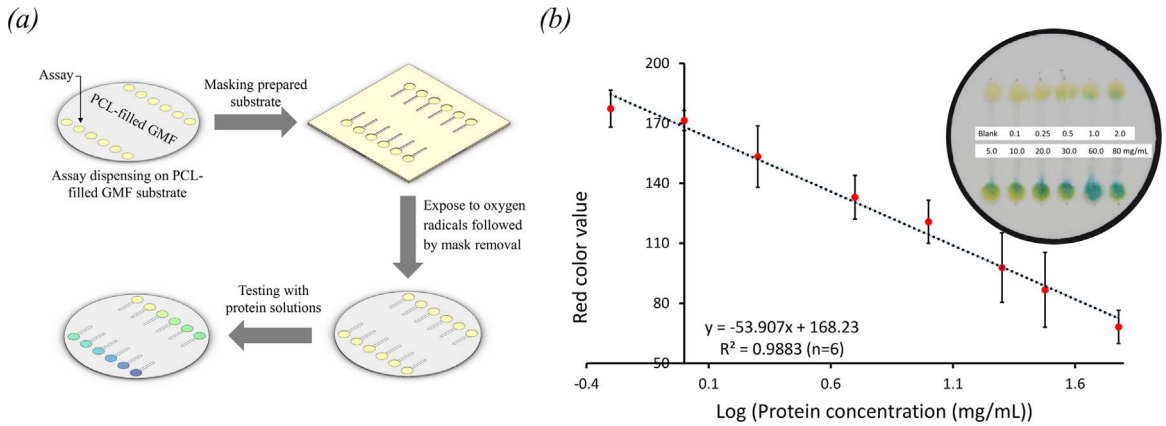

Fig. 4. Fabrication approach for production of a total protein analysis device on PCL-filled GMF membrane media (a). The device consists of 12 assay zones of 3 mm diameter circles. 3.27 µL of assay was evenly dispensed and dried over the assay zone before the fluidic channel fabrication. Oxygen radical exposure was done at 0.6 Torr pressure and 13 W Rf (fwd) power for 6 s (b) Calibration plot with red color channel on RGB color space. Insert: actual device used to measure a series of protein standards (top row: blank, 0.1, 0.25, 0.5, 1.0, 2.0 mg/mL; bottom row: 5.0, 10.0, 20.0, 30.0, 60.0, 80.0 mg/mL).

using the approach illustrated in Fig. 4a. To recap, a PCL-filled GMF membrane was placed on the platen of an HP D300 digital dispenser equipped with Bio Pattern software (HP, Corvallis, OR, USA) to selectively deposit reagents in user defined locations on the membrane. The reagents deposited consisted of 3.27 μL of a 2:1 mixture of pH 1.8 citrate buffer solution: TBB in 0.1% triton X. The prepared substrate was then masked using i tape which had previously been cut to the desired pattern using the laser cutter operated under optimized condition to obtain proper pattern dimensions. The required mask pattern was designed using SolidWorks drafting software. The mask was registered to the pre-patterned reagent areas by using printed fiduciary marks. The device thus prepared was placed face-up into the homebuilt oxygen plasma exposure system and exposed to oxygen radicals using the following settings: 13 W (forward) power, 0.6 Torr pressure, and 6 s. Exposure was accomplished using a homebuilt oxygen radical generator described in Section 2.3, comprising a commercially available oxygen plasma decontaminator. As decontaminators are optimized to generate long lived oxygen radicals rather than strong O_2 plasmas, and the exposure time is limited to few seconds, no assay chemistry change was observed following this step. However, it is also possible to dispense assay reagents after the fabrication of fluidic pathways if necessary (Fig. S9), and this approach would be ideal for more sensitive assay reagents such as enzymes. Removing the masking material revealed the completed, ready-to-use devices. Total time needed to produce a device was 43 min, including PCL treatment of GMF media (16 min), patterning of masks (2 min), deposition of reagents (22 min), masking and exposure (2 min), and mask removal (1 min). All processes are easily conducted in parallel, allowing for multiple devices to be made simultaneously. Cost per device (12 test zones), including reagents, is approximately US$ 1.00.

To use the device for total protein content analysis, 2.5 μL of sample (in this case, a standard solution or a human blood serum “unknown”) was applied to the sample inlet zone using a micropipette followed by 5 min of incubation at room temperature. Image based detection and digital red (R), green (G) and blue (B) color analysis was used in a quantitative detection approach. The intensity of the RGB color channels over the assay zone was measured (Fig. 4b insert) for each assay zone on the device. Both R and G color channels showed a good linear trend over the range of 0.5–60 mg/mL with respective R^2 of 0.988 and 0.989 as shown in Fig. S9(a). However, the R color channel was selected as the best fit color channel for quantitative detection as it exhibited higher sensitivity than the green channel. Normal human blood serum was tested in both undiluted and diluted (1:6) forms to probe the reliability of the assay in this microfluidic format. The diluted serum sample showed 10.58 mg/mL (R = 112.99 ± 9.37, n = 9) protein concentration while the undiluted sample showed a concentration of 61.84 mg/mL (R = 71.67 ± 9.44, n = 10). Both values are in agreement with the manufacturer's certified value of 62 mg/mL [31]. To further verify this result, the unknown serum sample was evaluated using a commercially available BCA protein assay kit (Biovision, Milpitas, CA, USA) in a well plate format. According to the BCA assay, the protein concentration in the human blood serum sample was 63.34 mg/mL (n = 3), again showing good agreement with the certified value as well as the determined value from the newly developed platform described herein. The calibration curve for the BCA protein assay kit is shown in Fig. S10(b). These results verify the accuracy and precision of the assay in this novel microfluidic format. Considering fabrication cost, feasibility and assay results, the performance of this device is superior to the performance of previously reported microfluidic devices using the same assay chemistry (Table S1).

4. Conclusions

Selective exposure of PCL-filled GMF membranes to oxygen radicals offers a new means of fabricating microfluidic devices. Selective exposure can be achieved using a mask made from inexpensive tape, allowing for two sided masking. This novel method allows for fabrication of complex multidimensional (2D and 3D) microfluidic devices simply by combining different channel geometries on the same polymer-filled membrane in a single exposure: an efficient and unique process. Depending on fabrication geometry, the channels will have unique properties and enable various unit operations such as microfluidic mixing, separations, delay and timing devices, etc. The performance of an immobilized colorimetric protein assay on this novel microfluidic platform demonstrated its practical applicability with different real world assay chemistries.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2017.08.031.

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