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Journal
Biomicrofluidics, 8(1)

ISSN
1932-1058

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Publication Date
2014

DOI
10.1063/1.4865675

Peer reviewed

Laminated microfluidic system for small sample protein analysis

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(Received 9 October 2013; accepted 2 February 2014; published online 13 February 2014)

We describe a technology based on lamination that allows for the production of highly integrated 3D devices suitable for performing a wide variety of microfluidic assays. This approach uses a suite of microfluidic coupons ("microfloupons") that are intended to be stacked as needed to produce an assay of interest. Microfloupons may be manufactured in paper, plastic, gels, or other materials, in advance, by different manufacturers, then assembled by the assay designer as needed. To demonstrate this approach, we designed, assembled, and characterized a microfloupon device that performs sodium-dodecyl-sulfate polyacrylamide gel electrophoresis on a small sample of protein. This device allowed for the manipulation and transport of small amounts of protein sample, tight injection into a thin polyacrylamide gel, electrophoretic separation of the proteins into bands, and subsequent removal of the gel from the device for imaging and further analysis. The microfloupons are rugged enough to handle and can be easily aligned and laminated, allowing for a variety of different assays to be designed and configured by selecting appropriate microfloupons. This approach provides a convenient way to perform assays that have multiple steps, relieving the need to design highly sophisticated devices that incorporate all functions in a single unit, while still achieving the benefits of small sample size, automation, and high speed operation. [http://dx.doi.org/10.1063/1.4865675]

I. INTRODUCTION

Microfluidics, which enables the manipulation of very small volumes of fluids in small devices, is seen as a promising technology for advancing areas of bio-analytical chemistry such as genomics and proteomics. Microfluidics offers the potential for shorter reaction times, lower reagent consumption, higher throughput, portability, versatility in design, and the possibility of integration with other systems.[1–6] Significant problems with microfluidic technology include the difficulty for manufacturing products that have dissimilar materials and technologies integrated in a single device, and for utilizing 3D designs. Manufacturing microfluidics tends to be monolithic and planar, requiring the development very clever or complex methods for building integrated devices.[7–10] Indeed, in most cases, additional functionality (such as pumping, filtering, imaging, electronics, etc.) is added externally to the chip since it is too difficult to integrate the required components on-chip.[11–14] Fluids are moved primarily in the plane of the device and assays tend to treat the device as a two-dimensional system. Moreover, chips tend to be completely sealed so that their assays must be predetermined at time of manufacture and cannot

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readily accommodate changes at the time of the assay or transport of material from one chip to another. These types of devices have been used with some success to replicate standard benchtop assays. In many cases, the assays have been cleverly and significantly redesigned so that they may work within the manufacturing limitations of the microfluidic system.[15,16]

This shortcoming in microfluidics manufacturing limits the types of assays that can be readily addressed. Assays that require a variety of different materials, processes, and transfer of analytes are particularly hard. An example of such as assay is the Western blot.[17] This assay starts with proteins prepared in a solution, which are then transferred to a gel medium for electrophoretic separation, and which are then blotted onto a solid membrane for immunochemical analysis. Since this assay is difficult to design and manufacture as a microfluidic system, clever workarounds have been developed such as embedding antibodies within the separation gel.[18–21]

Gel-based separations[22] and blotting may benefit from miniaturization to microfluidic scales. In particular, for applications that produce very small quantities of sample material, a small-sized assay is needed. An ideal Western-style micro-scale assay would be able to manipulate small volumes of sample, efficiently inject analytes into a very small zone, perform electrophoretic separations, allow separated species to be immobilized, and transferred to another system for further analysis such as immunostaining, high sensitivity imaging, or harvesting. Further, it would be useful if the details of the assay (such as staining antibodies, protocol, gel density, etc.) could be readily changed without the need for a completely new device.

To address this need, we propose a different approach to the design and production of microfluidic systems. In our approach, we produce microgels, microfluidics, and other functional microcomponents on thin paper carriers that are then laminated together to form a stack that can perform an assay (see Figure 1). We refer to each thin carrier as a "microfluidic coupon" or "microfloupon" for short. Microfloupons are typically manufactured on paper (although any material may be used). Paper is an ideal material for many microfloupons since paper is inexpensive, easy to modify, and cut, and has reasonable mechanical strength to enable handling. Moreover, the fibrous, porous nature of paper makes it convenient for embedding materials such as gels or polymers. For specialized functions (such as electronics or optics), microfloupons may be made differently from materials, such as polymers and metals. In the microfloupon approach, each microfloupon may be manufactured separately (provided by separate manufacturers if appropriate), then stacked together as needed to produce an assay of interest. If needed, the stack may be separated, and one or more of the microfloupons can be transferred to a new stack for further processing.

To illustrate the potential for microfloupons in microfluidic assays, we demonstrate the first half of a Western blot assay, sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), using small quantities of protein. This demonstration uses microfloupons to (1) manipulate a small sample of proteins, (2) inject them into a small zone, (3) perform gel electrophoretic separation, then (4) remove the gel from the system for imaging and further processing. The demonstration utilizes some features that are difficult to produce using

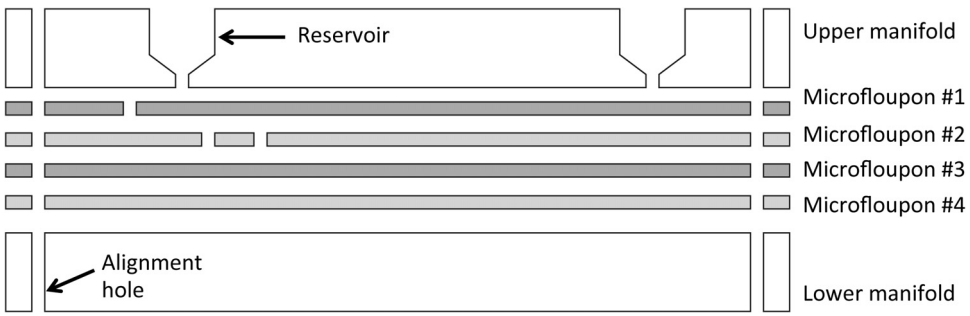

FIG. 1. General illustration of microfloupon laminate device. Multiple functional microfloupons form layers in a stack, allowing for 3-D integration of many dissimilar components. Microfloupons may contain microfluidics, thin gels, sieves/filters, electronics, optics, etc. Microfloupons may be mixed and matched as necessary to produce an assay, and may be moved from one stack to another.

conventional microfluidic technology. These include the use of an externally manufactured thin membrane to filter and hold proteins, the use of the vertical direction as a viable part of the assay, the use of a very thin acrylamide gel slab, and the ability to remove the gel after separation for further processing of the sample. All microfloupons used in the assay device were inexpensive and easy to manufacture.

II. EXPERIMENTAL

A. Chemicals, reagents, and materials

Pre-labeled Alexa Fluor 532 goat anti-rabbit IgG (composed of heavy and light chain proteins, 50 kDa and 25 kDa, respectively, lot#:1241449) and BenchMark^{TM} fluorescent protein standard (12 kDa, 23 kDa, 33 kDa, 41 kDa, 65 kDa, 100 kDa, 155 kDa lot#:1232327) were purchased from Invitrogen (Carlsbad, CA, USA). Acrylamide/bis-acrylamide solutions (29:1) 30% and 40%, 10x Tris–glycine–SDS buffer (25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS), SDS, tetramethylethylenediamine (TEMED), and ammonium persulfate (APS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Whatman track-etched membranes (0.1 μm lot#:65691, 0.08 μm lot#:49083, 0.015 μm lot#:54821) were donated by GE Healthcare (Waukesha, WI, USA). To make an appropriate polyacrylamide gel concentration (T), 30% and 40% (w/v) acrylamide-bis acrylamide was diluted with 4x lower Tris SDS electrophoresis buffer and water and when ready to use, proper amount of TEMED and APS were added to the total volume. For micropatterning work, Shipley 1827 photoresist, SU-8 developer, 1-methoxy-2-propyl acetate was obtained from Microchem Corp (Newton, MA). To produce 1002F resist, UVI-6976 photoinitiator (triarylsulfonium hexafluoroantimonate salts in propylene carbonate) was purchased from Dow Chemical (Torrance, CA), and γ-butyrolactone (GBL) solvent was purchased from Sigma Aldrich (St. Louis, MO).

To build the apparatus and other parts of the device, poly methyl methacrylate (PMMA) sheets were obtained from Anaheim Plastics (Anaheim, CA, USA) and polydimethylsiloxane (PDMS) polymer and PDMS curing agent (Sylgard 184 A/B) were purchased from Dow Corning (Elizabethtown, KY). For salinizing the surface of the glass, Sigmacote was purchased from Sigma-Adrich (St. Louis, MO, USA).

B. Design

The goal for this study was to demonstrate that a microfloupon stack can be used to perform SDS-PAGE analysis on a small sample of proteins. This involves manipulating proteins from a prepared sample, transport of the proteins into a small zone within a very thin polyacrylamide gel, electrophoretic separation of the sample in the thin gel, and removal of the gel with proteins intact for analysis and further processing.

To accomplish this, a stack was designed containing a top and bottom plastic manifold enclosure, and four microfloupons. These were (1) a thin polymer gasket/aperture microfloupon, (2) a paper-gel microfloupon, (3) a thin track-etched filter microfloupon, (4) a second paper-gel microfloupon. The design is shown in Figure 2. The details of the manufacturing of each component are described in Sec. II C; here, we focus on the design and function of the device.

The microfloupon stack was designed to be used with a rigid plastic enclosure on the top and bottom of the stack. The enclosure contained holes and channels machined in it and allowed for pipetting of reagent and sample into the system, routing of fluidic channels, attachment of electrical connections, and provided mechanical strength to the system. During operation, the entire stack (plastic enclosure and microfloupons) was placed in an aluminum fixture to hold everything and press the stack together. Alignment pins were used to make sure the microfloupons were aligned with respect to each other and the enclosure.

The system was designed to work as follows. Protein sample was pipetted into the top reservoir in the housing. Voltage was applied to the top and bottom electrodes, forcing the sample to be electrokinetically driven vertically from the reservoir through the top aperture microfloupon (1) into the running gel microfloupon (2). The current passed through the running gel,

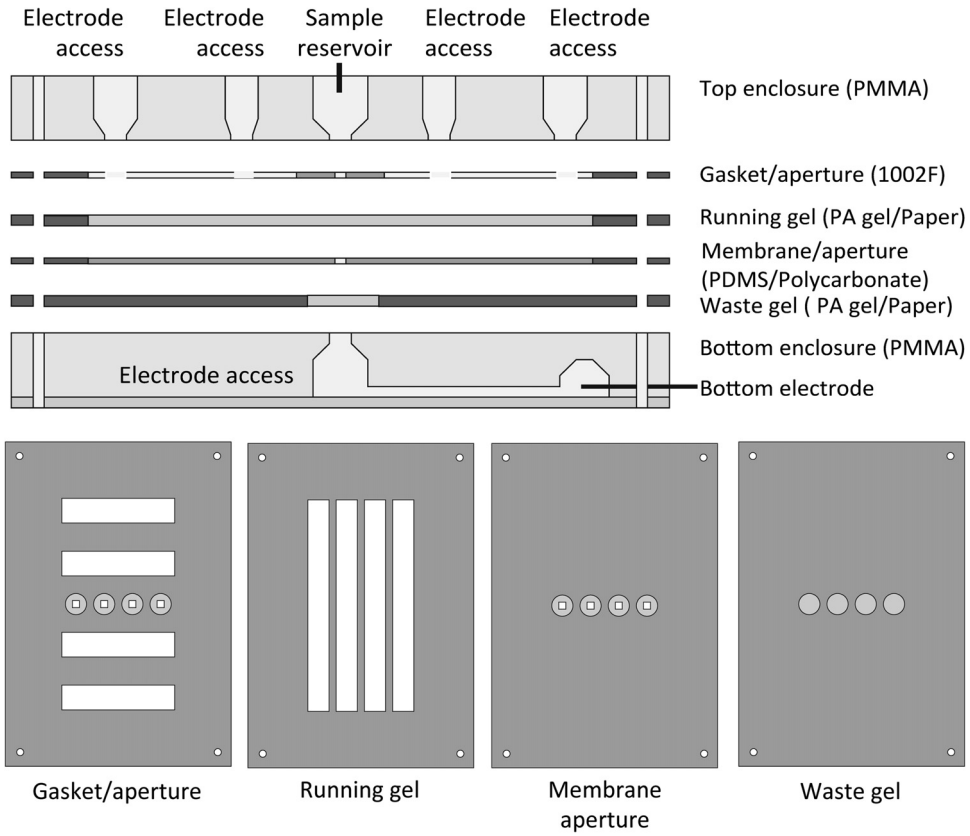

FIG. 2. Layout of microfloupons used for this study. The top enclosure had several holes and slots that were filled with buffer to allow electrical connection to the gel. The middle reservoir was used to load the sample, as well as for electrode access. The bottom enclosure also allowed electrical access through a hole and buffer; however, it was sealed to prevent buffer from falling out. The footprint for the entire structure was 4.3 cm × 7.0 cm × 1.5 cm.

through the track-etched membrane (3), and through the waste gel microfloupon (4) to the bottom of the device where the second electrode was placed. However, proteins were trapped by the track-etched membrane. After a suitable time, the voltage was switched to different electrodes, changing the electric field to a horizontal direction along the length of the running gel, and a standard electrophoresis separation was performed in the running gel.

Successful operation of the device relied on two phenomena: (1) shaping of the electric field lines within the running gel and (2) trapping the proteins at the surface of the track-etched membrane. For efficient operation, field lines had to be properly shaped to move the proteins into a small region of the gel without allowing dispersion to occur due to diverging field lines. Following focusing and injection of the proteins, the field lines were switched to run parallel to the running portion of the gel to allow for electrophoretic separation of the proteins.

Electric field lines are determined by the location of current sources and drains, and the arrangement of conductive and insulating media in the system. Figure 3 shows electric field simulations using 3D finite element analysis (COMSOL, Burlington, MA). Two configurations are shown. The first is a simple design containing a negative electrode in the reservoir and a second electrode below the gel to drive the proteins from the reservoir into the gel membrane. The second is the case where three negative electrodes were used with one positive electrode. In the gel microfloupon, gel was impregnated in both the main component of the paper and also in open lanes cut into the paper. The electrical impedance of the gel-filled paper was measured to be half that of the open gel regions, and this was used in the simulation of the electric field. Wide reservoirs at the electrode ends, and a parallel and symmetric design of the gel lanes is needed to ensure that field lines are parallel in the lanes.

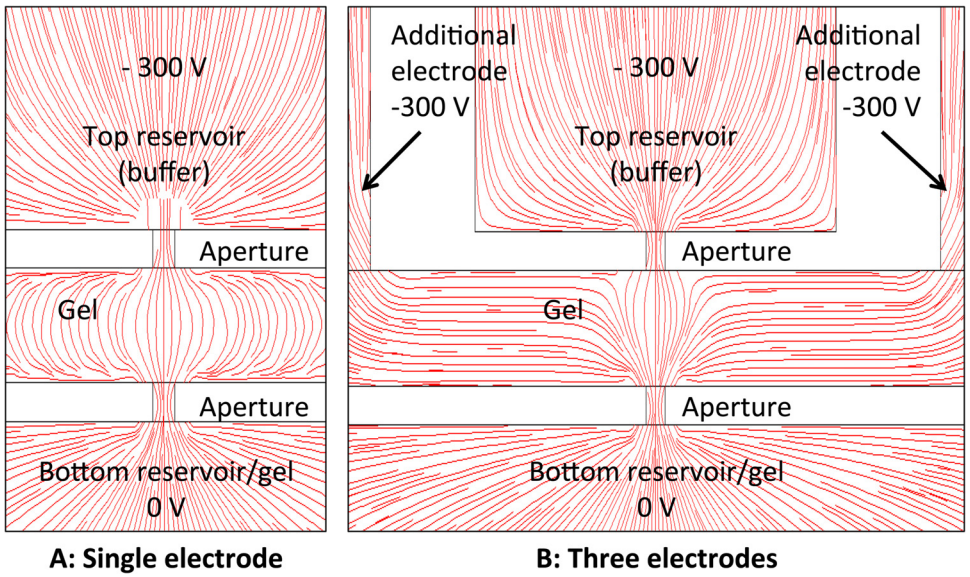

FIG. 3. Slice of 3D finite element analysis (COMSOL) simulation of electric fields on a representative electrophoresis device. Image (a) shows simulation results of field lines achieved using one electrodes with −300 V applied at the top and a grounded electrode at the bottom. Note the diverging of the field in the center of the gel. The second image (b) shows the concentration of electric field lines using three electrodes with −300 V applied to the top and sides and a grounded electrode the bottom.

A track-etched polycarbonate membrane was used to filter proteins while allowing ions and current to pass through. The membrane used 15 nm nanopores which appeared to block proteins in laboratory tests. Despite the large pore size of the membrane compared to typical protein size, our results show these to be reasonably effective in blocking the proteins. This may be due to the fact that polycarbonate is known to carry negative surface charge, which repels negatively charged SDS-coated proteins.[23]

C. Fabrication

The device consisted of a top and bottom plastic manifold enclosure, and four microfloupons: (1) a thin polymer gasket/aperture, (2) a paper-gel microfloupon, (3) a thin track-etched polycarbonate filter microfloupon, (4) and a second paper-gel microfloupon.

The plastic manifold enclosures (top and bottom) were cut from of slabs of PMMA (Anaheim Plastics, Anaheim, CA). Each piece was 4.3 cm × 7.0 cm × 6.35 mm thick. Holes, reservoirs, and channels were routed in the plastic using standard computer numerically controlled machining (CNC). Each enclosure had four precision through holes cut for alignment pins. These were used to align the top and bottom enclosures as well as the microfloupons between them. The plastic enclosures were used to provide a mechanical-fluidic enclosure, provide a flat surface to firmly hold the microfloupons together, and to allow easy electronic and fluidic access to the device through embedded the channels and reservoirs. The bottom manifold was sealed with a thin acrylic sheet to prevent fluid from falling out of the bottom channels. Acrylic was chosen for both enclosures because it is inexpensive, strong, easy to machine, and transparent in the visible range.[24]

The first microfloupon ("gasket/aperture") consisted of a thin polymer patterned with holes to let protein and electric current pass through from a reservoir in the top manifold to the acrylamide gel below it at a specific location. The polymer was a photo-definable epoxy, Photostructurable Resin PSR 1002F,[25] which allowed it to be manufactured as a thin film using standard photolithography. To manufacture the aperture layer, the uncured 1002F epoxy material was spun to a thickness of 100 μm on a glass surface. Spin speed was 500 rpm for 10 s followed by 30 s at 1200 rpm. The resin was then soft baked at 65 °C for 30 min to evaporate the

solvent. Using a computer generated photomask that defined small open regions, the material was exposed to UV light at 6 mW/cm^2 for 7 min to crosslink the polymer. SU-8 developer (MicroChem, Newton MA) was then used to remove the unexposed areas and develop the pattern. The film was removed from the glass surface by soaking it in distilled water (DI) water for 2–3 h. This film was then used as the first microfloupon in the stack, and could be aligned with the manifold by alignment notches placed in the plastic and in the microfloupon. The key feature of the gasket/aperture microfloupon was a small opening (100 μm × 100 μm) in the PSR 1002F, which allowed sample and electric current to pass into the running gel.

The second microfloupon ("running gel") was a polyacrylamide gel filled paper coupon designed to perform the function of collecting the proteins from the sample, then separating them along the length of the gel slab. This microfloupon was made from blot absorbent filter paper (Bio-Rad, Hercules, CA), 300 μm thickness. Four lanes, each 2 mm × 36 mm, were cut in the paper using a computer controlled CO_2 laser cutter (Versa Laser Systems, model VL200, Universal Laser Systems, Ltd, Scottdale AZ).

The microfloupon paper and the slots were filled with 12% polyacrylamide gel. Briefly, 30% acrylamide monomer (667 ml), SDS 4x lower buffer (500 μl), water (825 μl), and chemical initiators TEMED (1 μl), and APS (25 μl) were mixed together. This mixture was poured onto a paper microfloupon with pre-cut slots and allowed to soak into the paper. To ensure a flat gel of uniform thickness, the saturated paper microfloupon was placed between two clean glass surfaces and the polyacrylamide gel mixture was allowed to cure. To improve removal of the gel from the glass, the glass was first salinized by dipping in silicone solution (Sigmacote, Sigma-Aldrich, St. Louis, MO) for 15 min, rinsing and allowing to dry. The result after casting and pressing the polyacrylamide gel in the paper was a smooth, flat paper microfloupon that was impregnated with polyacrylamide gel. The slots contained thin slabs of pure polyacrylamide gel, approximately 300 μm thick, with almost no air bubbles or defects. Moreover, the microfloupon could be handled and easily assembled in the laminate stack without tearing the gel membrane.

The third microfloupon ("membrane/aperture") was a paper coupon impregnated with silicone PDMS and small openings in the center to allow current and ions to pass through to the bottom layer. A polycarbonate Whatman track-etched membrane (Whatman, Kent, UK) was patterned with photoresist to allow passage of current through a very small region and placed on the PDMS microfloupon before the PDMS had cured. When the PDMS cured, it held the membrane securely in place, while providing a good seal against leakage out the bottom and a transparent region for imaging.[26]

The track-etched membranes were 25 mm in diameter, 6 μm thick, and contained nanopores 15 nm in size. The membrane was prepared in order to produce a tiny region for the electrical current and ions to pass. It was first placed on a silicon wafer and held in place by polyimide tape. The wafer was then spin-coated using Shipley 1827 photoresist (MicroChem Corp, Newton, MA) for 40 s at 4000 rpm. After spinning, the membrane was baked at 95 °C on a hot plate for 3 min. A negative mask containing a pattern of 100 μm × 100 μm squares was placed on top of the membrane. The photoresist coated membrane was exposed to UV light (365 nm) at 11 mW/cm^2 intensity though the mask for 40 s. The membrane was then submerged in tetramethylammonium hydroxide (TMAH) developer for 40 s to develop the resist pattern, resulting in a precise opening that was patterned on the nanoporous membrane. After patterning, the photopolymer was fully cured by baking at 120 °C for 30 min. The non-exposed region of the membrane remained covered with the polymerized photoresist, thus sealing the pores everywhere except at the small aperture. To remove the membrane from the wafer, the wafer was soaked in DI water overnight. Figure 4 shows the results of this process.

The fourth microfloupon ("waste gel") was a paper coupon with a small opening that held 29% polyacrylamide gel in small openings, 2.5 mm in diameter. The percentage of the polyacrylamide gel was chosen to be over 22% which has the molecular weight (MW) cutoff of ~10 kDa.[27] The waste gel was chosen to be higher density to significantly slow down the migration of proteins leaving the system, thus allowing them to be imaged. This helped to understand the efficacy of the membrane for filtering the protein sample. This microfloupon

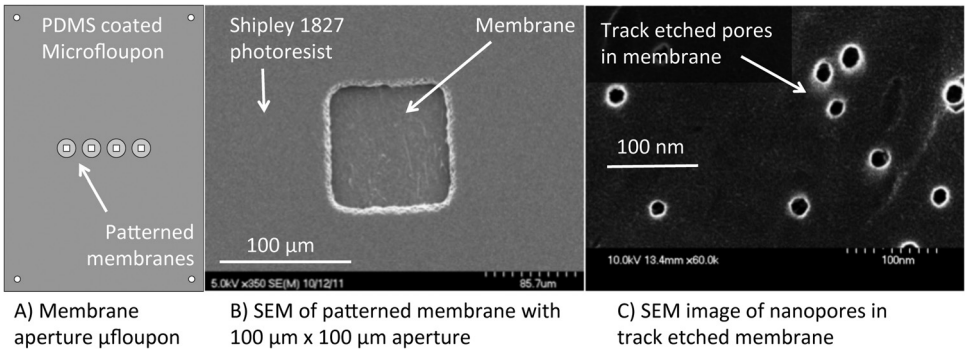

FIG. 4. Details of track-etched membrane patterned with photopolymer to define small apertures. (a) Microfloupon containing four apertures with membranes. (b) Scanning electron micrograph of aperture defined by photopolymer, enabling placement of precision opening on the membrane. (c) Close-up image of nanopores in the track-etched membrane.

also contained a PDMS coating, which was manually applied and cured that served to act as a bottom gasket for the system. The purpose for this microfloupon was to provide an electrically conductive path to the bottom enclosure, and to capture any proteins that might have passed through the membrane. This floupon was made in a similar manner to the running gel. A small amount of gel solution was pipetted into the holes and allowed to cure using the same protocol as the running floupon. The gel easily impregnated part of the paper, providing a secure holding for the gel after it cured.

The bottom plastic enclosure had holes drilled to correspond with the openings in the waste gel microfloupon. These holes led to a channel that in turn led to a large cavity that contained a platinum electrode, which was inserted and sealed through a hole in the side of the enclosure. The channel was filled with running buffer, which provided electrical connection from the microfloupon gel to the electrode. The cavity contained a small pore to allow fluid loading and to let air escape if electrolysis occurred during operation.

The entire stack, consisting of two plastic enclosures and four microfloupons, was placed in a fixture to press all the layers together, forming a single multi-structured device that could be used to perform loading, concentrating, and electrophoretic separating of proteins. After completing the separation step, the stack could be separated and any of the microfloupons removed for further processing.

D. Experiment

To test the device, a standard SDS-PAGE assay was performed using a protein ladder in one of the four lanes. Two studies were performed, as illustrated in Figure 5. The first study (loading and injection) demonstrated that proteins could be injected into a small region within the acrylamide gel. The second study (separation) demonstrated that proteins could be electrophoretically separated in a thin microfloupon gel and that the microfloupon could be removed from the lamination stack with gel and protein undisturbed, and that fluorescent analysis could be done on the microfloupon directly.

For both studies, prior to the experiments, the microfloupons were stacked and sandwiched between the plastic enclosures. The top and bottom reservoirs and channels were filled with 1 x SDS buffer (pH 8.3), taking care to avoid formation of bubbles. For the bottom enclosure, a syringe was used to fill the enclosed channel and cavity through a small hole in the side. This hole was also used to insert a bottom platinum electrode wire. Electrodes were connected to a high voltage power supply HP6209B (Hewlett-Packard, Palo Alto, CA).

The first study looked at the protein loading and injection and is illustrated in Figure 5. For this study, 10 ng Alexa Fluor 532 labeled goat anti-rabbit IgG(H + L) protein was added to 200 μl of running buffer in the top reservoir. A platinum electrode was placed in the reservoir, as well as two electrodes in the side openings for field shaping purposes. −330 V was applied to the top electrodes with respect to the bottom ground electrode. The electric field was applied

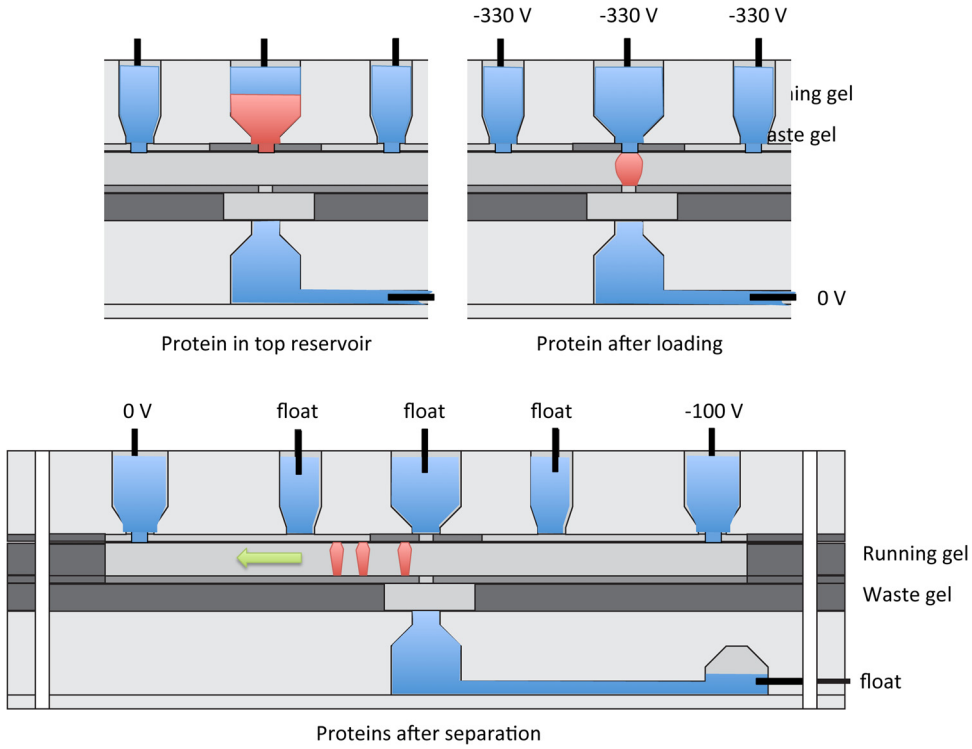

FIG. 5. Basic procedure for studies. For the loading study (top), labeled protein sample was loaded into reservoir above gel. After electric field was applied, protein sample was allowed to electrophoretically migrate into the running gel. After loading, running gel and waste gel were imaged for proteins. For separation study, after loading, the voltages were switched to electrodes at the ends of the running gel and protein sample was allowed to separate. After separation, the running gel microfloupon was removed and imaged. Drawings are for illustration only. Components are not to scale, and gel thicknesses have been exaggerated for clarity.

for 2 min, 6 min, 8 min, and 12 min allowing the proteins to electromigrate into the gel layer. After each experiment, the gel microfloupon was removed, then imaged under an Olympus IX71 inverted microscope equipped with fluorescein isothiocyanate (FITC Ex (494 nm)-Em (518 nm)) filters. In addition, the gel was cut using a surgical blade to reveal the cross section of the gel where the migration of the protein was expected. The gel was sandwiched between plastic blocks and turned up for imaging under the same microscope. This procedure allowed cross sectional images to be taken. Together the images indicated the ability of this system to focus proteins into a small plug suitable for electrophoretic separation.

To determine if the loading and injection process was efficient, the same imaging procedure was performed for the waste gel to determine how much of the protein passed through the membrane and left the main gel. Presumably, after some time, proteins may have migrated out of the running gel and passed through the track-etched membrane, thereby reducing the loading efficiency in the running gel. However, those proteins should be trapped within the acrylamide waste gel that was below the membrane, allowing them to be imaged.

In addition, after each experiment, the fluid in the reservoir was removed and analyzed for remaining protein. To do this, nitrocellulose (NC) blotting membrane along with filter papers were soaked in tris-buffered saline and Tween-20 (TBS-T) solution and placed in a Bio-Rad 96 well bio-dot apparatus (microfiltration vacuum blotting device). The apparatus was sealed using vacuum pressure. As a control, a 10 ng protein sample diluted in 200 μl running buffer was dispensed into one well of the bio-dot apparatus. The liquid samples collected from each experiment were similarly transferred, and all wells were washed with TBS-T solution to ensure that all of the protein in the sample was adsorbed on to the NC membrane. Using vacuum, the liquid contents of the wells was filtered through the NC and the wash repeated three times. The NC membrane was then air-dried in the dark and imaged using a fluorescent microscope

(Olympus IX71) to quantitate the amount of protein sample left in the injection reservoir during the loading and injection steps. To account for background noise, the signal derived from an image of a well with no protein sample was subtracted from all of the results, including the control.

The second study examined protein separation and imaging. This proceeded in the same manner as the first study, except that after loading, the electrodes were moved to the ends of the device, and the central electrode was not used. At this time, −100 V was applied between the first electrode and the second electrode. This initiated electrophoretic migration along the length of the running gel.

III. RESULTS AND DISCUSSION

A. Protein loading and injection

Injection experiments were performed for 2, 6, 8, and 12 min. A constant voltage of −330 V was applied to force proteins into the gel, and current was monitored during the injection. The highest power consumption was 67.4 mW and it dropped to 33.7 mW after 12 min. Heating effects were not observed during the trials. Figure 6 shows the imaging results from the loading and injection study.

Cross-sectional images in Figure 6 clearly show the movement of the proteins into the running gel, with the highest concentration starting at the top and advancing to the bottom of the gel over time. During injection, the protein front appeared to move in a well behaved manner, consistent with the known electric field geometry. One can see that the plug shape matches the expected shape from the electric field simulations, as shown in Figure 7. Furthermore, at long injection time (30 min) the proteins appeared to pile up on the membrane at the bottom of the gel.

Most of the protein was driven out of the loading reservoir and into the gel membrane. Results of analysis of the reservoir solution are shown in Figure 8. By 8 min, very little of the loaded protein could be detected in the reservoir by quantitation of filtration adsorption (Figure 8, lower panel), suggesting that most of the proteins had left the solution.

The system relied on the use of a track-etched membrane to block proteins at the surface, while simultaneously allowing current and ions to pass through. However, since the pores were 15 nm, it is possible that proteins (which are typically smaller than this), would not be stopped by the membrane but rather pass through the pores. Some blocking of the proteins may be expected since the polycarbonate membranes are negatively charged. A 29% polyacrylamide gel microfloupon was placed under the track-etched membrane microfloupon to capture proteins that passed through the membrane. Figure 9 shows the results of imaging of these gels after

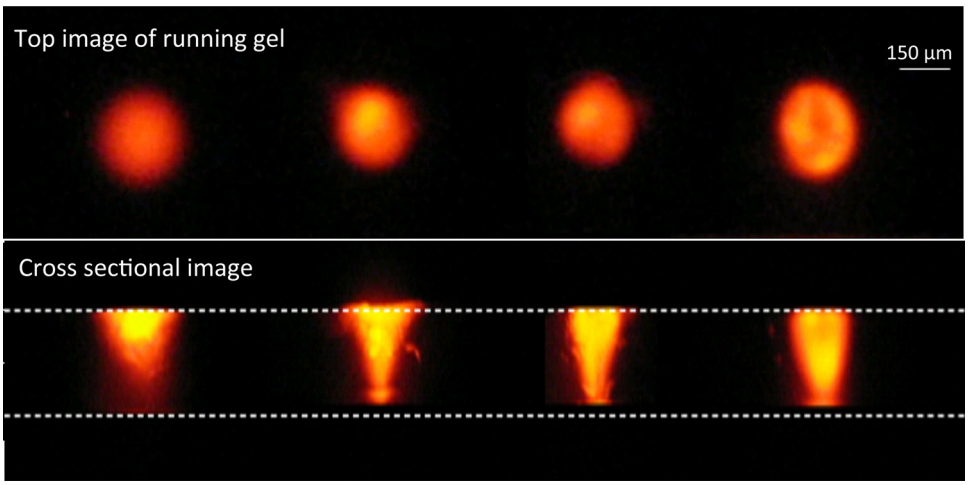

FIG. 6. Imaging of protein sample after loading and injection into the running gel. Results from the 4 loading durations (2, 6, 8, and 12 min) are stitched together, from left to right, respectively. The running gel microfloupon as removed from the stack and imaged after each experiment. Cross sectional image was obtained by cutting the protein gel and imaging the side.

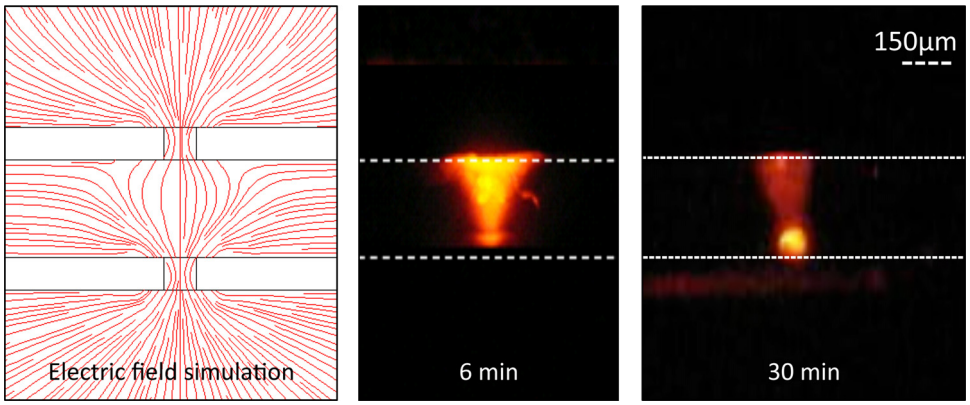

FIG. 7. Comparison of finite element analysis of field lines (taken from Figure 3) with cross sectional image of proteins during injection (taken from Figure 6). The experimental results indicate the field shaping causes the proteins to pinch together in the gel. Also shown at far right is a cross-sectional image taken after 30 min of injection. In this case, most of the protein migrated to the bottom of the gel.

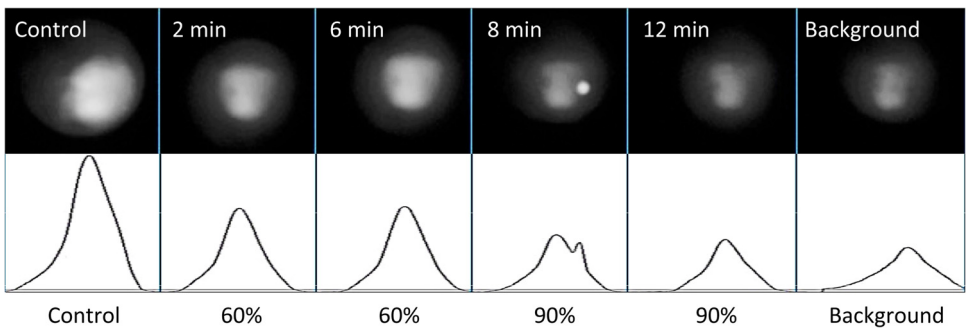

FIG. 8. Analysis of fluid remaining in reservoir after loading into running gel. Top panel: Image of protein remaining in the loading well, adsorbed onto NC. Bottom panel: Most of the proteins are missing from the reservoir after 8 min of injection (imaging is close to background). These results are mostly qualitative, but indicate that the electromigration of proteins out of the well is reasonably efficient.

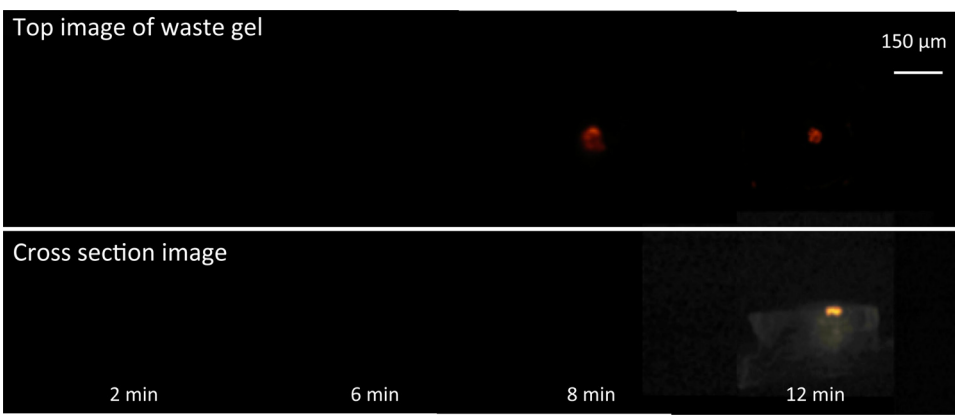

FIG. 9. Imaging of waste gel (under track-etched membrane) after loading and injection into the running gel. The waste gel microfloupon was removed from the stack and imaged after each experiment. Cross sectional image was obtained by cutting the protein gel and imaging the side. After 8 min, some protein was seen to pass through the membrane into the gel below.

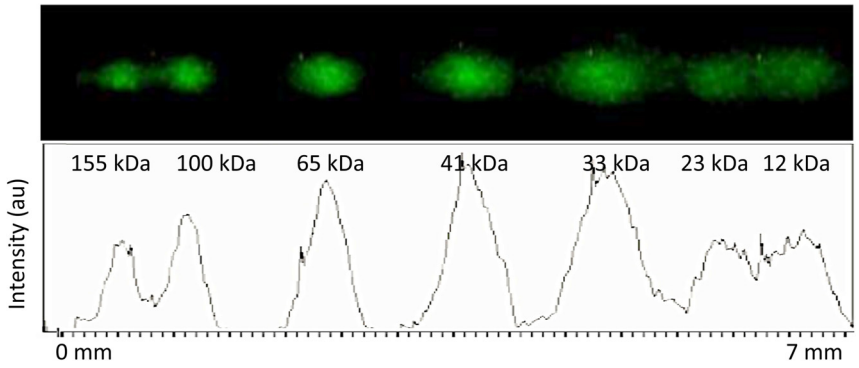

FIG. 10. Imaging of running gel after separation experiment. All seven bands of the protein ladder are visibly separated in the gel. The intensity of the small proteins appears lower than the other proteins which may be an indication that the smaller proteins are leaking through the track-etched membrane, and being depleted from the injection plug. This separation occurred within 7 mm.

different injection times. After 8 min of injection, the proteins can be seen in the gel indicating that some proteins had indeed passed through the membrane. Thus, while the track etched membranes may have been effective and slowing down the rate of protein loss, they did allow a small fraction of proteins to pass through. Therefore future assays that wish to use this strategy would need to use smaller pore membranes, other ionic membranes, or use careful timing of the injection step to ensure efficient injection of the proteins into the gel.

B. Protein separation

The second study looked at the ability for a microfloupon system to perform a protein separation and to image the proteins in their microfloupon, out of the device. To do this test, a separation experiment was performed using a "ladder" containing 7 fluorescently tagged proteins ranging in size from 12 kDa to 155 kDa. A 500 nl protein sample was loaded into the injection reservoir and injected for 12 min using the method described. Then the voltages on the electrodes were changed, switching the direction of electric field to the horizontal direction, resulting in electrophoresis of the sample. During separation mode, the anode and cathode electrodes were connected to wide conductive paths at each end (Figure 2). This feature, combined with the symmetric and parallel design of the gel lanes, resulted in parallel electric fields along the length of the gel microfloupon, despite the fact that the gel lanes have higher conductivity than the gel filled paper. This was confirmed with full 3D analysis of the electric fields. Thus, no cross-talk in the current between lanes was expected.

The electrophoresis was run for 3 min, the running gel microfloupon was removed from the stack and imaged. Since the microscope field of view was not wide enough to capture all the protein samples in a single image, the microfloupon was imaged at different points and the image was merged. Figure 10 shows the 7 protein bands of the known standards, indicating that proteins as small as 12 kDa can be collected on the membrane and separated along the gel of the microfloupon, using the SDS-PAGE microfloupon device.

All seven protein bands were clearly identified in the gel, indicating that the system was well behaved and that the protein separation worked according to expectations. Moreover, since the microfloupon was readily removed from the device, it could be readily imaged using conventional imaging tools such as a fluorescent microscope. The gel microfloupon was easy to handle and was not torn or distorted during disassembly or transport, despite being approximately 300 μm thin. For example, the gel microfloupon containing the proteins could be placed on a blotting device, if desired, or placed in a high sensitivity imager.

IV. CONCLUSION

In this study, we demonstrated the development and utility of a general-purpose laminate technology that utilizes microfluidic coupons to integrate multiple materials and technologies

together into a small platform for doing micro-scale assays. The microfloupons are manufactured separately, then laminated together to form a final integrated device that can perform a bioassay on a small size scale. In this work, our microfloupons were made from gel and paper, silicone rubber, track-etched membrane, and photo-sensitive polymer, resulting in low cost laminates with high functionality. We demonstrated this technology for a gel-based application (gel electrophoresis). Gels are typically difficult to integrate into microfluidic devices, and layer-to-layer functionality is particularly difficult. However, with the microfloupon approach, gels are readily integrated, and functionality between layers (such as use of apertures, electrical vias, filtration) is readily included.

The application described in this paper was for injecting and separating proteins in a gel, following the SDS-PAGE protocol. Injection was accomplished through the use of shaping electric fields, apertures built into the microfloupons, and a nanoporous membrane built into a microfloupon. We demonstrated that 10 ng of protein can be collected into a small plug in the microfloupon with reasonable efficiency. We used a track-etched membrane, which slowed down the protein transport in the gel, but ultimately did not capture all the proteins. However, it did allow us to explore the use of protein injection using field shaping and lithographic patterning of apertures, such as the use of a 100 μm aperture patterned directly on the track-etched membrane. Future versions of this strategy should use membranes with smaller pore sizes, or use ionic membranes such as dialysis membranes—both of which are hard to integrate into conventional microfluidics, but easy to incorporate into a microfloupon stack.

By changing the direction of the electric fields, we demonstrated that injection could be followed by electrophoretic separation of the proteins, with protein masses ranging from 12 kDa to 155 kDa. The gel microfloupon was easily removed, handled, and transported to another system for imaging. If desired, this gel microfloupon could be placed in a second cassette for electroblotting, suggesting that this system can be designed for a full Western analysis with small protein samples. A major benefit of the microfloupon approach is that different technologies can be combined together as needed to produce an assay of interest.

This work demonstrates that a new paradigm can be pursued for integrating microfluidics that is low cost, highly flexible, and easy to use. The microfluidic coupon approach greatly simplifies manufacturing and integration of microfluidic devices, while still allowing for the benefits of miniaturization. Moreover, microfloupons are relatively easy to manufacture by conventional precision manufacturing approaches, and thus should scale nicely to mass production.

AKNOWLEDGMENTS

The authors gratefully acknowledge the financial contribution of the NSF IGERT LifeChips Award #0549479, DOED GAANN in LifeChips Award #P200A120220, and ARCS Foundation. Also we would like to thank GE Healthcare for their generous donation of track-etched membranes. We thank Richard Chang for experimental assistance and we thank Lily Wu for her help in this research.

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