A World-to-Chip Interface for Digital Microfluidics

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Digital microfluidics (DMF) is a fluid handling technique that enables manipulation of discrete droplets on an array of electrodes. There is considerable enthusiasm for this method because of the potential for array-based screening applications. A limitation for DMF is nonspecific adsorption of reagents to device surfaces. If a given device is used to actuate multiple reagents, this phenomenon can cause undesirable cross-contamination. A second limitation for DMF (and all other microfluidic systems) is the "world-to-chip" interface; it is notoriously difficult to deliver reagents and samples to such systems without compromising the oft-hyped advantages of rapid analyses and reduced reagent consumption. We introduce a new strategy for digital microfluidics, in which a removable plastic "skin" is used to (a) eliminate cross-contamination and (b) bridge the world-to-chip interface. We demonstrated the utility of this format by implementing on-chip protein digestion on immobilized enzyme depots. This new method has the potential to transform DMF from being a curiosity for aficionados into a technology that is useful for biochemical applications at large.

Microfluidics is a multidisciplinary field of study characterized by the use of integrated devices to manipulate fluids in micrometer-length dimensions.[1,2] While microfluidics is typically implemented in microchannels, an alternative paradigm has recently emerged, called digital microfluidics (DMF). In this technique, droplets of fluid are manipulated across a surface of patterned electrodes by means of electromechanical forces.[3–7] Droplets on DMF devices are isolated from their surroundings rather than being embedded in a stream of fluid, a simple method of forming microreactors in which there is no possibility that products will diffuse away. Perhaps more importantly, in DMF, each sample can be addressed individually,[8] this stands in contrast to the related technique of manipulating droplets in microchannels.[9]

There is currently great enthusiasm for applying DMF to a wide variety of applications including cell-based assays,[10] enzyme assays,[11–14] protein sample preparation,[15–18] and the polymerase chain reaction.[19] Unfortunately, there are two critical limitations on the scope of applications compatible with DMF, biofouling and interfacing. The former limitation, biofouling, is a pernicious one in all microscale analyses; a negative side-effect of high surface area to volume ratios is the increased rate of adsorption of analytes from solution onto solid surfaces. We[20] and others[12,21] have developed strategies to limit the extent of biofouling in digital microfluidics, but the problem persists as a roadblock, preventing wide adoption of the technique.

The second limitation for DMF (and for all microfluidic systems) is the "world-to-chip" interface; it is notoriously difficult to deliver reagents and samples to such systems without compromising the oft-hyped advantages of rapid analyses and reduced reagent consumption.[22,23] A solution to this problem for microchannel-based methods is the use of preloaded reagents. Such methods typically comprise two steps: (1) reagents are stored in microchannels (or in replaceable cartridges) and (2) at a later time, the reagents are rapidly accessed to carry out the desired assay/experiment. Two strategies have emerged for microchannel systems; in the first, reagents are stored as solutions in droplets

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isolated from each other by plugs of air[24] or an immiscible fluid[25,26] until use. In a second, reagents are stored in the solid phase in channels and are then reconstituted in solution when the assay is performed.[27-29] Preloaded reagents in microfluidic devices is a strategy that will be useful for a wide range of applications.[2] Until now, however, there has been no analogous technique for digital microfluidics.

In response to the twin challenges of nonspecific adsorption and world-to-chip interfacing in digital microfluidics, we have developed a new strategy relying on removable polymer coverings.[30,31] We call these coverings "skins" to highlight the central concept; after each experiment, a thin film is replaced, but the central infrastructure of the device is reused. The skins effectively prevent cross-contamination between repeated analyses and, perhaps more importantly, serve as a useful medium for reagent introduction onto DMF devices. To demonstrate this principle, we preloaded dried spots of enzymes to skins (which we call "skin depots") for subsequent use in proteolytic digestion assays. The loaded reagents were found to be active after >1 month of storage in a freezer. As the first technology of its kind, we propose that this innovation may represent an important step forward for digital microfluidics, making it an attractive fluid-handling platform for a wide range of applications.

EXPERIMENTAL SECTION

Reagents and Materials. Angiotensin I and II, bradykinin, bovine α-chymotrypsin and trypsin, proteinase K, myoglobin, ubiquitin, rhodamine B, Tris-HCl, CaCl₂, trifluoroacetic acid (TFA), Pluronic F127, and acetonitrile were purchased from Sigma-Aldrich Canada (Oakville, ON). 20mer DNA oligonucleotide (5′AGCAGAGCGACCT-CAATGAT3′) was purchased from Sigma Genosys (Oakville, ON). An Enzchek protease assay kit (including labeled, quenched BODIPY-casein) and FITC-labeled bovine serum albumin (FITC-BSA) were obtained from Invitrogen (Burlington, ON). Ultramarker was obtained from Thermo Fisher Scientific (Ottawa, ON). α-Cyano-4-hydroxy-cinnamic acid (α-CHCA), 2,5-dihydroxybenzoic acid (DHB), 3-hydroxypicolinic acid (HPA), and sinapinic acid (SA) were purchased from Waters Limited (Mississauga, ON). Parylene-C dimer was from Specialty Coating Systems (Indianapolis, IN), and Teflon-AF was from DuPont (Wilmington, DE). 1-Ct silicone oil (vapor pressure = 4 mm Hg at 25 °C) was purchased from Gelest Inc. (Morrisville, PA). Polyethylene food wrap with the trademark "Saran" was purchased from grocery stores, and plastic tape was from Grand & Toy (Toronto, ON).

Clean room reagents and supplies included Shipley S1811 photoresist and MF321 developer from Rohm and Haas (Marl-

borough, MA), AZ300T photoresist stripper from AZ Electronic Materials (Somerville, NJ), solid chromium from Kurt J. Lesker Canada (Toronto, ON), CR-4 chromium etchant from Cyantek (Fremont, CA), and concentrated sulfuric acid and hydrogen peroxide (30%) from Fisher Scientific Canada (Ottawa, ON). Piranha solution was prepared as a 3:1 (v/v) mixture of sulfuric acid and hydrogen peroxide.

Working solutions (10 mg/mL) of all matrixes (α-CHCA, DHB, HPA, and SA) were prepared in 50% analytical grade acetonitrile/deionized (DI) water (v/v) and 0.1% TFA (v/v) and were stored at 4 °C away from light. Stock solutions (10 μM) of angiotensin I, II, and bradykinin were prepared in DI water, and stock solutions (100 μM) of ubiquitin and myoglobin were prepared in working buffer (10 mM Tris-HCl, 1 mM CaCl₂ 0.0005% w/v Pluronic F127, pH 8). All stock solutions of standards were stored at 4 °C. Stock solutions (100 μM) of digestive enzymes (bovine trypsin, α-chymotrypsin, and proteinase K) were prepared in working buffer and were stored as aliquots at −80 °C until use. Immediately preceding assays, standards and enzymes were warmed to room temperature and diluted in DI water (peptides) or working buffer (proteins, enzymes, and fluorescent reagents). Fluorescent assay solution (3.3 μM quenched BO-DIPY-casein and 2 μM rhodamine B in working buffer) was prepared immediately prior to use.

Device Fabrication and Operation. Devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) fabrication facility using processes similar to those reported previously.[20,30] Briefly, glass slides were cleaned with piranha solution (15 min) and then coated with 200 nm of chromium by electron beam deposition. The substrates were primed by spin-coating with HMDS (3000 rpm, 30 s) after rinsing and baking on a hot plate (110 °C, 5 min) and were then spin-coated with Shipley S1811 photoresist (3000 rpm, 30 s). Substrates were prebaked on a hotplate (100 °C, 5 min) and exposed through a photomask using a Suss Mikrotek mask aligner. Substrates were developed in MF321 (3 min) and then postbaked on a hot plate (100 °C, 3 min). After photolithography, substrates were immersed in chromium etchant (1 min). Finally, the remaining photoresist was stripped in AZ300T (10 min).

Prior to experiments, devices were fitted with unmodified polymer coatings formed from food wrap (called "skins") or reagent-loaded polymer coatings (called "skin-depots"). Unmodified skins were prepared by adhering coverings to unpatterned glass substrates, spin-coating with Teflon-AF, (1% w/w in Fluorinert FC-40, 1000 rpm, 60 s), and postbaking on a hot plate (75 °C, 30 min). When ready for use on digital microfluidic devices, a few drops of silicone oil were dispensed onto an electrode array, followed by application of the skin (Teflon-AF side up), followed by annealing on a hot plate (75 °C, 2 min). Skin-depots were prepared as above, but prior to use, they were modified by application of reagent spots. This step was achieved by pipetting 2 μL droplet(s) of enzyme (6.5 μM trypsin, 10 μM α-chymotrypsin, or 13 μM proteinase K) onto the Teflon-coated surface and allowing them to dry. Skin depots were either used immediately or were sealed in a plastic Petri-dish and stored at −20 or −80 °C. Prior to use, skin depots were allowed to warm to room temperature (if necessary), peeled off of the unpatterned substrate, applied to a silicone-oil coated electrode array, and annealed on a

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hot plate (75 °C, 2 min). In addition to food wrap, plastic adhesive tape and wax film (Parafilm M, Alcan Packaging, Neenah, WI) were also evaluated for use as digital microfluidic device skins. Skins formed from tape were attached to devices by applying gentle pressure, while those formed from wax film were manually stretched (to about 10 μm thickness) and then wrapped around the device to make a tight seal free of air bubbles.

Devices had a "Y" shape design of 1 mm × 1 mm electrodes with interelectrode gaps of 10 μm. Droplets of 2 μL were moved and merged on devices operating in the open-plate mode (i.e., with no top cover) by applying driving potentials (400−500 V_{rms}) to sequential pairs of electrodes. The driving potentials were generated by amplifying the output of a function generator operating at 18 kHz and were applied manually to exposed contact pads. Droplet actuation was monitored and recorded by a CCD camera.

Mass Spectrometry. Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to evaluate samples actuated on DMF devices. Matrix/sample spots were prepared in two modes: conventional and in situ. In the conventional mode, samples were manipulated on a device, collected with a pipet, and dispensed onto a stainless steel target. A matrix solution was added, and the combined droplet was allowed to dry. In the in situ mode, separate droplets containing sample and matrix were moved, merged, and actively mixed by DMF and then allowed to dry onto the surface. In in situ experiments involving skin depots, matrix/crystallization was preceded by an on-chip reaction: droplets containing sample proteins were driven to dried spots containing digestive enzyme. After incubation with the enzyme (room temperature, 15 min), a droplet of matrix was driven to the spot to quench the reaction and the combined droplet was allowed to dry. After cocrystallization, skins were carefully peeled off of the device and then affixed onto a stainless steel target using double-sided tape. Different matrixes were used for different analytes: α-CHCA for peptide standards and digests, DHB for ultramarker, HPA for oligonucleotides, and SA for proteins. At least three replicate spots were evaluated for each sample.

Samples were analyzed using a MALDI-TOF Micro-MX MS (Waters, Milford, MA) operating in the positive mode. Peptide standards and digests were evaluated in the reflectron mode over a mass to charge ratio (m/z) range from 500−2 000. Proteins were evaluated in linear mode over a m/z range from 5 000−30 000. At least 100 shots were collected per spectrum, with laser power tuned to optimize the signal-to-noise ratio (S/N). Data were processed by normalization to the largest analyte peak, baseline subtraction, and smoothed with a 10-point running average. Spectra of enzyme digests were analyzed using the Mascot protein identification package searching the SwissProt database. The database was searched with one allowed missed cleavage, a mass accuracy of ± 1.2 Da, and no further modifications.

Fluorescence. Confocal fluorescence microscopy was used to evaluate protein adsorption on surfaces, using methods similar to what we reported previously.[20] Briefly, before and after a droplet containing FITC-BSA (7 μg/mL) was driven across a device (four times), the surface of the skin was imaged using a Fluoview 300 scanning confocal microscope (Olympus, Markam, ON) equipped with an Ar$^+$ (488 nm) laser, in conjunction with a 10× objective

(NA 0.95) (Figure 1a). Fluorescence was passed through a 510−525 nm band-pass filter, and each digital image was formed from the average of four frames using FluoView image acquisition software (Olympus).

A fluorescence plate reader was used for long-term skin depot stability assays. Skin depots were thawed and affixed onto devices, and a 2 μL droplet containing the assay reagent (quenched BODIPY-casein) and internal standard (IS) (rhodamine B) was driven to the dried spot by DMF. After reaction for 30 min in a humidified chamber, the device was positioned on the top of a 96-well plate (with the droplet aligned to one of the wells) and inserted into a PheraStar multiwell plate reader (BMG Labtech, Durham, NC). The droplet was evaluated by sequential measurements for BODIPY-casein signal (λ_{ex} = 485 nm; λ_{em} = 520 nm) and IS signal (λ_{ex} = 530 nm; λ_{em} = 600 nm). Five replicates (using five separate skin depots) were evaluated for each storage period (1, 2, 3, 10, 20, or 30 days), and the data were evaluated for statistical significance using a two-tailed student's t test.

RESULTS AND DISCUSSION

Protein Adsorption and Cross-Contamination. As is the case for most substrates (e.g., pipet tips, centrifuge tubes, multiwell plates, etc.), digital microfluidic devices are prone to nonspecific adsorption of proteins. For example, as shown in Figure 1a, after a droplet containing FITC-BSA is translated on a DMF device, a residue is left on the surface that can be detected by confocal microscopy. Such residues can cause two types of problems for DMF: (1) the surface may become sticky, which impedes droplet movement, and (2) if multiple experiments are to be performed, cross-contamination may be a problem.

An example of evidence for cross-contamination on a DMF device is shown in Figure 1b,c. In this experiment, two different analytes (10 μM angiotensin I in the first run, 1 μM angiotensin II in the second) were evaluated by MALDI-MS after actuation across the same path on the same device. As shown, the spectrum of angiotensin I generated after the first run (Figure 1b) is clean; however, the spectrum of angiotensin II (Figure 1c) is contaminated with residue from the previous run. In these tests, after actuation by DMF, the sample droplets were transferred to a MALDI target for crystallization and analysis, meaning that the cross-contamination comprised both (a) an adsorption step from the droplet to the surface in the first run and (b) a desorption step from the surface to the droplet in the second run. These processes must occur rapidly, as the droplets are in contact with the surface for a short period of time. This unpredictable and rapid adsorption and desorption (and the ensuing cross-contamination) was the initial motivation for the work reported here.

Digital Microfluidic Skins. To overcome the phenomenon of nonspecific adsorption, we developed a new strategy for DMF, which facilitates unlimited use of a given device. As depicted in the cartoon in Figure 2, this strategy comprises replacing the permanent insulating layer used in conventional DMF devices with a removable "skin." In this format, reactions and assays are carried out as usual, by applying sequences of electrical potentials to merge and mix reagent droplets. As is the case for conventional DMF, reagent molecules tend to adsorb to the surface (depicted in Figure 2, step 3, as colored dots). After the assay is complete, the skin can be peeled off from the device and replaced with a
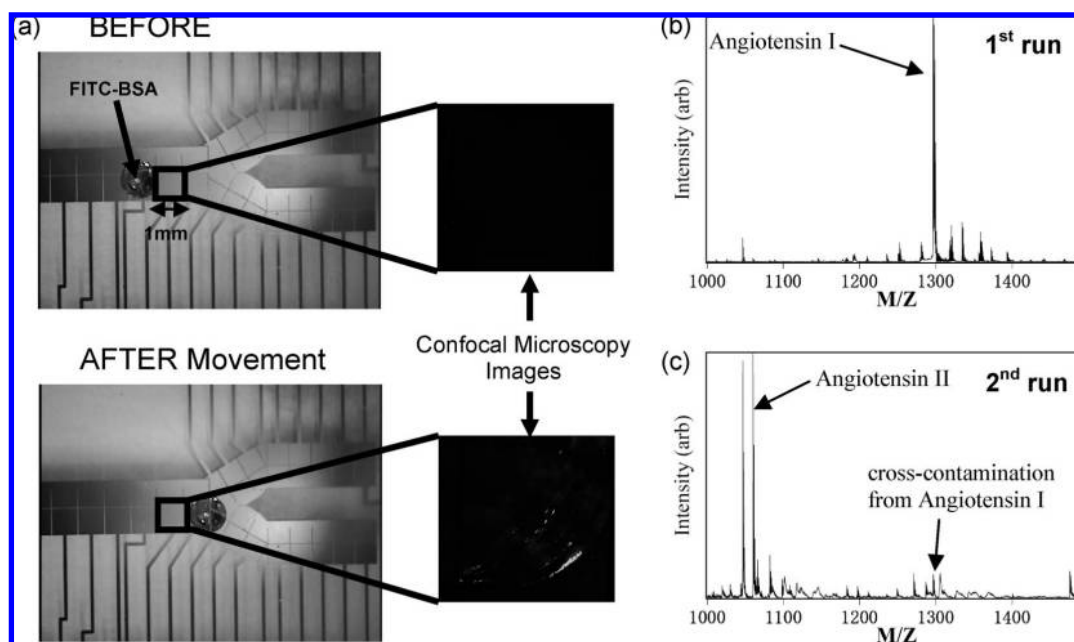
Figure 1. Images and spectra demonstrating protein adsorption and cross-contamination in digital microfluidics. In part a, the top image shows a device prior to droplet actuation, paired with a corresponding confocal fluorescence image of a central electrode. The bottom image shows the same device after a droplet containing FITC-BSA (7 μg/mL) has been cycled over the electrode four times, paired with a corresponding confocal image. The two fluorescence images were processed identically. The spectra in parts b and c were generated from two different droplets manipulated on the same digital microfluidic device (the first and second runs, respectively). The spectrum generated in the first experiment (b) is of 10 μM angiotensin I (MW 1296); the spectrum generated in the second experiment (c) is of 1 μM angiotensin II (MW 1046). In part c, cross-contamination from the first run is observed.
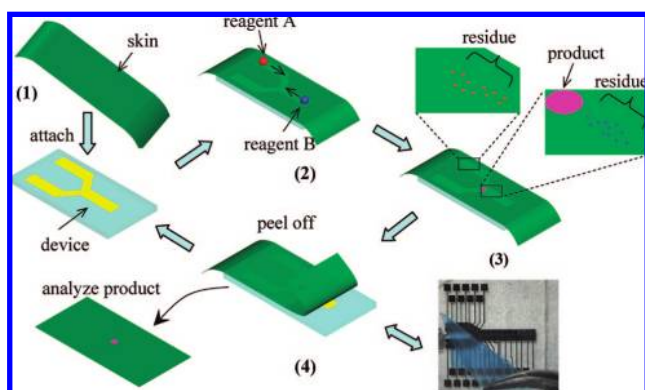

Figure 2. Schematic depicting the removable skin strategy. (1) Fresh piece of polymer covering is affixed to a DMF device; (2) reagent droplets are actuated on top of the covering to facilitate mixing and merging; (3) residue is left behind as a consequence of nonspecific adsorption of analytes; (4) the plastic covering is peeled off and the product can be analyzed if desired (a picture of a device with a skin formed from adhesive tape is shown in the bottom right). This process can be repeated ad nauseum by using additional skins.

fresh one for subsequent use. Thus, the device can be used indefinitely by eliminating cross-contamination. In addition, for assays that involve the formation of dried products (e.g., cocrystallization with matrix for MALDI-MS[16−18]), the skins serve as a convenient analysis tool; the product of a given assay can be analyzed while at the same time the device is reused for subsequent assays.

To illustrate the new strategy, four different types of analytes were processed on a single DMF device, using a fresh skin for each run. As shown in Figure 3, the four analytes included insulin (MW 5733), bradykinin (MW 1060), a 20-mer oligonucleotide

(MW 6135), and the synthetic polymer, Ultramark 1621 (MW 900−2200). Each skin was analyzed by MALDI-MS in situ, and no evidence for cross-contamination was observed. In the work described here, a relatively simple Y-shaped electrode design was used (suitable for one-step processes); however, we also confirmed that the skin strategy is compatible with more complex electrode arrays appropriate for multistep reactions. This is important because the more complex the design, the more care is required in fabrication. In our laboratory, DMF devices are typically disposable (used once and then discarded); however, in experiments with removable skins, we regularly used devices for 9−10 assays with no drop-off in performance. Thus, in addition to eliminating cross-contamination, the skin strategy significantly reduces the fabrication load required to support DMF.

In these experiments, we evaluated three different types of substrates for forming skins, polyethylene food-wrap sheets (∼15 μm thick), clerical adhesive tape (∼45 μm), and stretched sheets of wax film (∼10 μm). Each of these materials was found to support droplet movement and facilitate device reuse. Skins formed from food-wrap were the most reliable and were used to collect the data reported here; however, a drawback for this material is the requirement of thermal annealing prior to use. In contrast, skins formed from tape and wax films did not require an annealing step, which made them convenient to use. Other concerns, however, made these materials less attractive. Skins formed from adhesive tape tend to damage the actuation electrodes after repeated applications (although this would likely not be a problem for low-tack tapes, which were not tested here). In addition, the thick tape substrates required the application of larger driving potentials (∼900 V$_{rms}$) for droplet manipulation. In contrast, the thin stretched wax films were compatible with
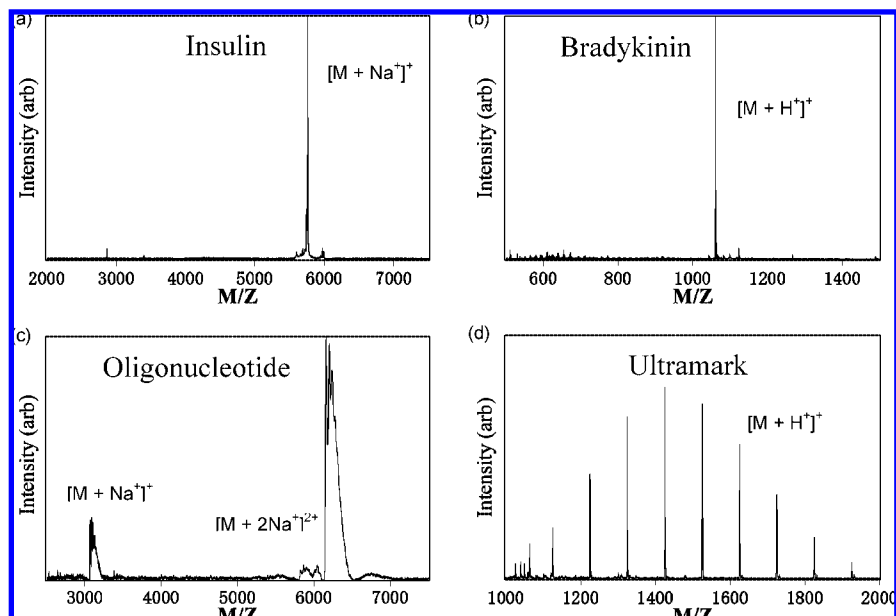
Figure 3. MALDI-MS analysis of different analytes processed on different skins using a single DMF device: (a) 35 μM insulin (MW 5733), (b) 10 μM bradykinin (MW 1060), (c) 10 μM oligonucleotide (MW 6135), and (d) 0.01% ultramark (MW 900−2200).
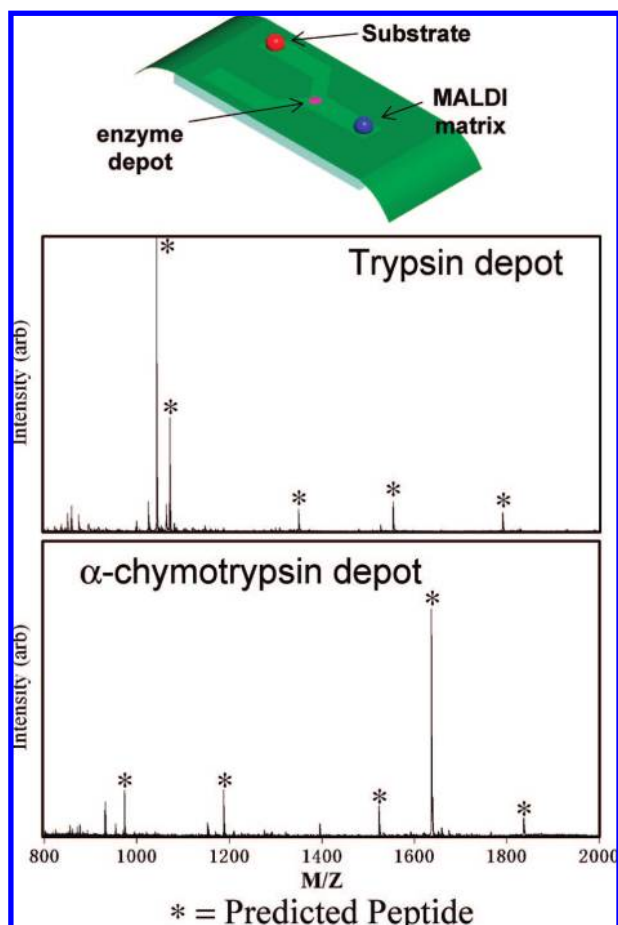

Figure 4. Schematic and spectra demonstrating the skin depot strategy. The spectra were generated from ubiquitin samples digested on prespotted trypsin (top) and α-chymotrypsin (bottom). Peaks labeled with asterisks (∗) were identified via a database search using Mascot, and the sequence coverage for all analyses was found to be greater than 50%.

driving potentials similar to those used for skins formed from food wrap. However, the thickness of skins formed in this manner was observed to be nonuniform, making the skins less reliable for droplet movement. Regardless, these preliminary results suggest that a variety of different materials (e.g., thin films of silicone or other plastics) may be compatible with the removable skin concept.

Two potential pitfalls for the removable skin strategy are trapped air bubbles and material incompatibility. In initial experiments with skins formed from food-wrap, bubbles were occasionally observed to become trapped between the skin and the device surface during application. When a driving potential was applied to an electrode near a trapped bubble, arcing was observed, which damaged the device. We found that this problem could be overcome by moistening the device surface with a few drops of silicone oil prior to application of the plastic film. The oil evaporates upon annealing, leaving a bubble-free seal. The latter problem, material incompatibility, might be a concern if materials in the skin were found to leach into solution, which could interfere with assays. The Teflon coating makes this unlikely, and in our experiments, no contaminant peaks were observed in any MALDI-MS spectra (including in control spectra generated from bare skin surfaces, not shown). We cannot rule out the possibility of this being a problem in other settings, but given the apparent wide range of materials that can be used to form skins (see above), we are confident that alternatives could be used in cases in which Teflon-coated polyethylene food wrap is not tenable.

Skin Depots. In exploring the replaceable skin strategy to overcome fouling and cross-contamination, we realized that this method could, in addition, serve as the basis for an exciting new innovation for digital microfluidics. By predeposition of reagents onto skins (and by having several such substrates available), the skins are transformed into a convenient new platform for rapid introduction of reagents to a device. Thus, this "skin depot" strategy is a potential solution to the well-known world-to-chip interface problem for microfluidics.[22,23]
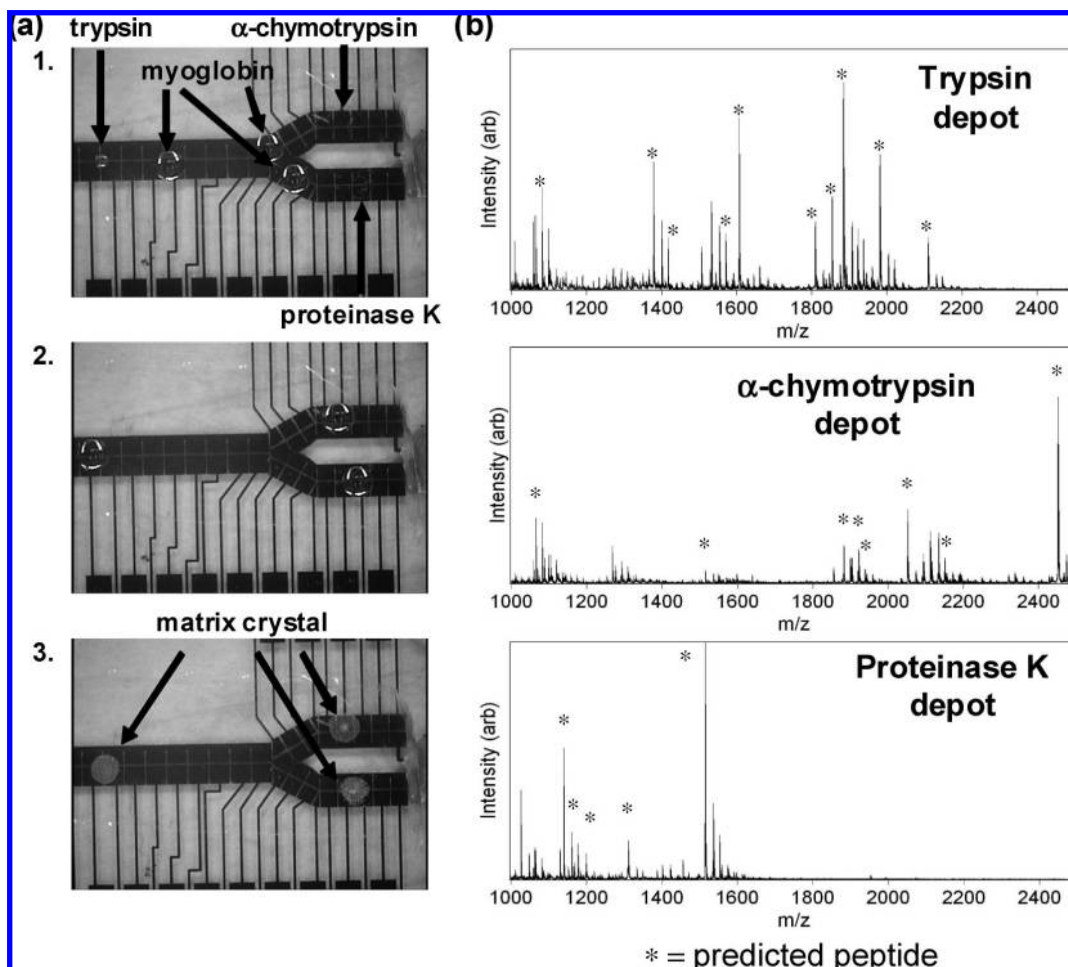
Figure 5. Multiplexed skin depot. (a) A series of pictures from a movie (top-to-bottom) demonstrating a skin depot analysis in parallel. Droplets of myoglobin (panel 1) were delivered to enzyme depots and allowed to incubate (panel 2), followed by delivery of matrix and drying onto the skin (panel 3). (b) Spectra were generated from myoglobin samples digested on prespotted trypsin (top), α-chymotrypsin (middle), and proteinase K (bottom). Peaks labeled with asterisks (∗) were identified via a database search using Mascot, and the sequence coverage for all analyses was found to be greater than 50%.

To illustrate the new strategy, we prepared skins loaded with dry enzyme depots and then used DMF to deliver droplets containing analytes and MALDI matrixes to the spots for analysis. In one type of experiment, illustrated in Figure 4, each skin was loaded with a single enzyme, and multiple assays were carried out sequentially by exchanging the skins. This strategy would be appropriate for cases in which an end-user wants to rapidly switch between different analyses for different samples. In a second type of experiment, illustrated in Figure 5, each skin was loaded with multiple enzymes and assays were carried out in parallel. This strategy would be appropriate for cases in which an end-user wants to carry out multiplexed assays on a given sample. While these proof-of-principle experiments comprise a small number of reagents, we speculate that in the future, a microarray spotter could be used to form skins appropriate for high-throughput analysis. Regardless, the performance of the proteolysis assays reported here was excellent; when evaluated using the proteomic search engine Mascot, sequence identification was 50% or more for all cases.

In optimizing the skin depot strategy for protease assays, we observed the method to be quite robust despite a number of potential pitfalls. First, pluronic F127 was used as a solution additive to facilitate movement of the analyte droplets (in this case,

ubiquitin and myoglobin); this reagent has been shown to reduce ionization efficiencies for MALDI-MS.[32] Fortunately, the amount used here (0.0005% w/v) was low enough such that this effect was not observed. Second, enzymatic autolysis peaks were only rarely observed, which we attribute to the low enzyme-to-substrate ratio and the short reaction time. Third, in preliminary tests, we determined that the annealing step (75 °C, 2 min) did not affect the activity of dried enzymes. In the future, if reagents sensitive to these conditions are used, we plan to evaluate skins formed from materials that do not require annealing (such as low-tack tape). Regardless, the robust performance of these first assays suggests that the strategy may eventually be useful for a wide range of applications, such as immunoassays or microarray analysis.

As described, the skin depot strategy is similar to the concept of preloaded reagents stored in microchannels.[24–29,33,34] This is a powerful approach for technology translation, as it spares the end user from having to procure the reagents and interface them

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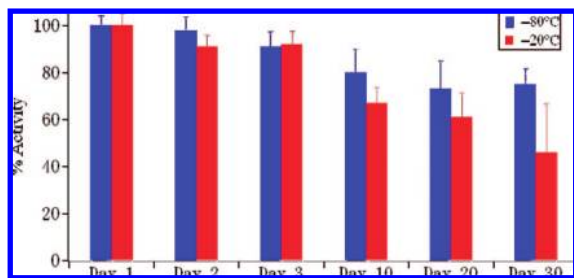
Figure 6. Skin depot stability assay: The fluorescence of protease substrate (BODIPY-casein) and an internal standard were evaluated after storing skin depots for 1, 2, 3, 10, 20, and 30 days. The substrates were stored at −20 °C (red) or −80 °C (blue). The mean response and SD were calculated for each condition from five replicate skins.

with the experimental setup. Unlike these previous methods, in which devices are typically disposed after use, in the skin depot strategy the fundamental device architecture can be reused for any number of assays. Additionally, because the reagents (and the resulting products) are not enclosed in channels, they are in an intrinsically convenient format for analysis. For example, in this work, the format was convenient for MALDI-MS detection, but we speculate that a wide range of detectors could be employed in the future, such as optical readers or acoustic sensors.

Skin Depot Stability. To be useful for practical applications, preloaded reagents must be able to retain their activity during storage. To evaluate the shelf life of skin depots, we implemented a quantitative protein digest assay. The reporter in this assay, quenched BODIPY-labeled casein, has low fluorescence when intact but becomes highly fluorescent when digested. In skin depot assays, a droplet containing the reporter was driven to a preloaded spot of trypsin, and after incubation the fluorescent signal in the droplet was measured in a plate reader (as described previously[10,11,20]). In preliminary experiments with freshly prepared skins, it was determined that at the concentrations used, the reaction was complete within 30 min. An internal standard (IS), rhodamine B, was used to correct for alignment errors, evaporation effects, and instrument drift over time.

In shelf life experiments, skins were stored for different periods of time (1, 2, 3, 10, 20, or 30 days) at −20 or −80 °C. In each experiment; after thawing the skin, positioning it on the device, driving the droplet to the trypsin, and incubating for 30 min, the reporter/IS signal ratio was recorded. At least five different skins were evaluated for each condition. As shown in Figure 6, shelf life performance was excellent; substrates stored at −80 °C retained >75% of the original activity for periods as long as 30 days. Substrates stored at −20 °C retained >50% of the original activity over the same period. The difference might simply be the result of different average storage temperature or might reflect the fact that the −20 °C freezer was used in autodefrost mode (with regular temperature fluctuations), while the temperature in the −80 °C freezer was constant. Regardless, the performance of these substrates was very encouraging for a first test, and we anticipate that the shelf life might be extended in the future by adjusting the enzyme suspension buffer pH or ionic strength or by adding stabilizers (e.g., disaccharides used widely for dry protein preservation[35]).

CONCLUSION

We have developed a new strategy for digital microfluidics, relying on removable plastic coverings, or skins. This strategy facilitates virtually unlimited reuse of devices without concern for cross-contamination, as well as enabling rapid access to preloaded reagents. As a first solution to the world-to-chip interface problem for the digital microfluidic format, this innovation has the potential to make this technology attractive for a wide range of applications.

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