Lab on a Chip

RSC Publishing

HIGHLIGHT

View Article Online
View Journal | View Issue

Research highlights

Cite this: *Lab Chip*, 2013, **13**, 1991
DOI: 10.1039/c3lc90037a

www.rsc.org/loc

Imee G. Arcibal,[a] Donald M. Cropek,[a] Mehmet R. Dokmeci[bc] and Ali Khademhosseini*[bcde]

Rapid, multiplexed protein analysis

Protein analysis is vital for a variety of disciplines and applications ranging from disease diagnosis and treatment schemes to the fundamental understanding of cellular and biochemical processes. Western blotting is an analytical tool capable of yielding useful information about proteins based on molecular mass and antibody binding. Conventional Western blotting, however, with macroscale slab gels and blotting membranes is limited by its low throughput, resource intensive nature, and multiple time-consuming and labour-intensive steps, requiring several pieces of equipment. Previous attempts on miniaturization have suffered from limited separation resolution between proteins,[1] low protein capture efficiency,[2] and complex interface and device architectures.[3]

In an attempt to address these issues, Herr and colleagues have elegantly combined the steps of protein sizing and immunoaffinity probing on a single microfluidic platform (μWestern)[4] based on their enabling advances in photopatternable light activated polymers.[3] The μWestern employs a unique photoactive gel (PACTgel) capable of functioning as both a sieving gel and a blotting polymer contained within microchannels of 48 distinct blots with the small footprint of a standard 1-inch by 3-inch microscope slide. Each of these 48 blots consists of three identical microchannels, which serve as triplicates for a total of 144 analyses on a single chip. The protein analysis occurs in three stages: (1) single microchannel protein sizing, (2) in-chip protein blotting by photocapture, and (3) antibody probing (Fig. 1). During the initial protein sizing, the PACTgel electrophoretically separates proteins using a large-to-small pore-size discontinuity following the initial preconcentration event utilizing an integrated electrode array. Proteins are subsequently immobilized in place *via* UV irradiation of benzophenone groups within the gel, which causes covalent bonding between the gel and the proteins. Once the proteins are immobilized, fluorescently tagged antibodies are delivered through the microchannels to interact with and bind to target analytes.

Transcription factor NFκB from cell lysate samples, as well as several purified HIV proteins, were used as model analytes to compare the performance of the μWestern platform and conventional Western blotting procedures. For both studies, the μWestern platform was not only able to achieve the same results as the conventional assay, but did so in less time (60 min *versus* 6–8 h) and required far less reagents (<1 ng *versus* ~1 μg of antibody and ~300 μL *versus* 300 mL buffer). Moreover, the μWestern allowed for protein quantification in the low pM range.

Additionally, the authors interrogated their platform with human sera to search for HIV antibodies. Serum poses additional challenges as a sample medium as it contains a variety of confounding proteins, which can potentially interfere with the separation and probing of the target proteins during a Western blot analysis. Although there was nonspecific staining by the antibody probes, likely leading to the

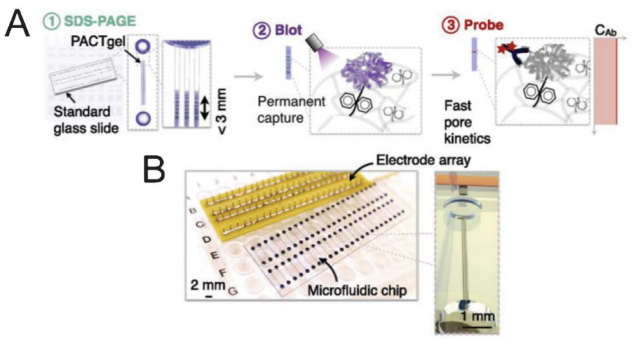

Fig. 1 A schematic representation of the μWestern assay. (A) Stages of the assay: (1) Protein sizing with PACTgel in a sieving mode; (2) Modified blotting with protein capture by the PACTgel; (3) Fluorescent antibody probing of bound proteins. (B) The footprint of the assay on a standard microscope slide. Figure adapted and reprinted with permission from Hughes *et al.*[4]

[a]*US Army Corps of Engineers Construction Engineering Research Laboratory, Champaign, Illinois 61822, USA*
[b]*Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts 02139, USA. E-mail: alik@rics.bwh.harvard.edu*
[c]*Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*
[d]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA*
[e]*World Premier International-Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, Sendai 980-8577, Japan*

observed increase in background signal, the μWestern easily differentiated among strongly reactive, weakly reactive, and non-reactive controls. This technology advances us closer to the coveted ''medic-on-a-chip'' for intensive assays that currently require substantial clinical laboratory infrastructure (*i.e.*, confirmatory diagnostics). Translating Western blotting to a microfluidic format represents an application that truly exploits the benefits of the microscale, including low mass transport times, low fluidic and analyte requirements, and the ease of multiplexing.

In addition to clinical diagnostics, the μWestern platform has the potential to revolutionize current protein analysis paradigms. With the ability to analyze 48 samples in triplicate simultaneously within an hour time frame, researchers could study the effects of different gene mutations, drug treatments, pathogen/toxin exposures, *etc.*, on protein levels at the single cell/small population level to gain greater insight into the mechanisms behind these changes at more time points and with additional experimental variations. These insights allow more conclusive correlations between protein presence and levels, and the normal/abnormal functions of cells, tissues, organs, and entire organisms at the systems level.

High throughput isolation of mammalian clonal colonies

The ability to create genetically engineered mammalian cells capable of expressing particular phenotypes has great implications for understanding the function and interactions of various proteins. The isolation of these cells once generated, however, requires labour-intensive and time-consuming protocols even before the cells can be characterized and identified using cell-destructive assays. Despite advances in the culture and manipulation of mammalian cells with the advent of microscale devices,[5,6] facile, high throughput selection and collection of engineered cells remains elusive.

In their recent article,[7] Allbritton and co-workers describe an innovative microarray device able to facilitate the growth of isolated single cells into clonal colonies for further proliferation and subsequent studies. The device takes advantage of two aligned arrays – the pallet array for cell seeding and initial colony growth, and the printing array onto which a subset of the colony's cells is transferred to examine cell phenotypes and intracellular protein expression *via* destructive analyses. As seen in Fig. 2, the cells are first seeded on the pallet array (A, B) prior to transfer to the corresponding posts of the printing array (C, D). The authors employed registration marks to facilitate the precise alignment of the cuboid structures of the pallet array with the corresponding posts on the printing array, allowing them to easily correlate the results obtained from the cells on the printing array with the original colonies on the pallet array.

To optimize the interaction between the two arrays, Gach *et al.*[7] investigated how the diameter and the height of the print array posts affected cell transfer efficiency. They

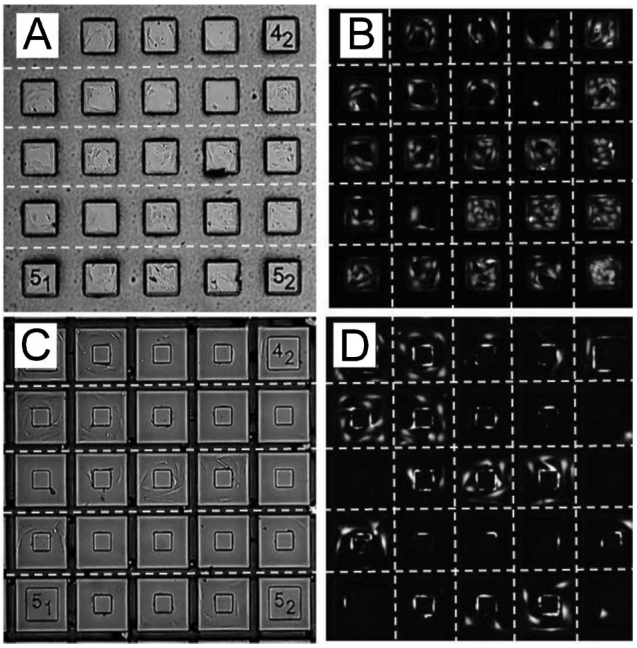

Fig. 2 Culture and printing of HeLa cells. Bright-field (A) and fluorescent (B) images of the pallet array with corresponding bright-field and fluorescent images of cells transferred to the corresponding printing array (C, bright-field; D, fluorescence). Fluorescence is due to viability staining with Calcein Red–Orange. Figure adapted and reprinted with permission from Gach *et al.*[7]

determined that smaller post diameters were more efficient for transferring the cells from the pallet to printing arrays with greater than 85% cell transfer for 30 μm and 60 μm posts, while a significant decrease in efficiency occurred for larger, 100 μm posts. The use of bigger post heights (100 μm) was also found to increase cell transfer efficiencies. The authors postulate that the taller posts were able to enclose more fluid between the two arrays, increasing the nutrients and oxygen available for the trapped cells. Additionally, it was observed that multiple cell types could be moved between arrays with printing rate efficiencies between 78% and 92%, despite differences in morphology and tissue origin.

The pallet and the corresponding printing arrays were investigated as a platform for the identification and isolation of IA32 cells engineered to express GFP, while silencing the Coronin 1B protein, which plays a key role in innate immune function. Following the initial seeding of the engineered cells onto the pallet array, cells transferred to the printing array were subjected to antibody staining to identify those that exhibited complete Coronin 1B knockdown. Subsequently, the corresponding pallets containing the desired phenotypic cells were laser ablated from the surface of the array and allowed to grow on a glass substrate. After four days, 40% of the collected pallets were found to contain cells with both GFP expression and silenced Coronin 1B, while the remaining pallets either had no cell proliferation or recovered Coronin 1B expression. Engineered cells evaluated by traditional fluorescence activated cell sorting (FACS) resulted in only 6% of the wells generating viable cell colonies, which could only be analysed

for protein expression by Western blot following four weeks of growth.

The pallet and printing scheme developed by the Allbritton group offers both high throughput and expedient analysis of engineered mammalian cells. The utility of this platform lies not only in its ability to quickly isolate and identify cells of interest, but also in the maintenance of a stable culture of the cells without the sacrifice of the majority of the initial test subjects for destructive phenotypic analysis. The rapid establishment of engineered cell lines will further fundamental studies concerning protein function, as well as the creation of cell-based sensing schemes capable of reacting to the presence of pathogens, toxins, and toxicants.

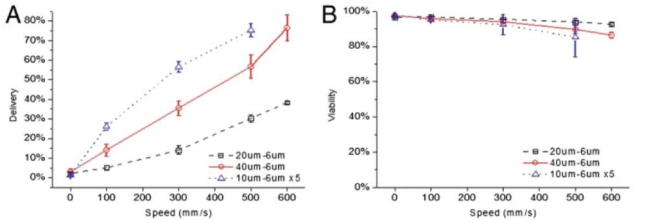

Fig. 3 Delivery performance depends on cell transport speed and constriction design. Constriction dimensions are denoted by numbers (*e.g.*, 10 μm–6 μm × 5) such that the first number corresponds to constriction length, the second to constriction width and the third (if present) to the number of constrictions in series per channel. (A) Delivery efficiency and (B) cell viability 18 h after treatment as a function of cell speed for 40 μm–6 μm (○), 20 μm–6 μm (□), and 10 μm–6 μm × 5 (△) device designs. Figure adapted and reprinted with permission from Sharei *et al.*[9]

Intracellular payload delivery

The literature is replete with techniques to introduce, inject, or implant a macromolecular payload into cells for diverse applications in sensing, metabolic reprogramming, therapeutics, and molecular tracking. Current methods, however, are limited by necessary chemical modifications of the payload, low throughput, low efficiency and treated cell viability, target molecule size restrictions, and recalcitrant cell types. Jensen and co-workers have overcome many of these issues by introducing a novel microfluidic system based on the generation of transient membrane openings in cells by their rapid deformation in narrow channels.[8] The authors have expanded on this initial work to demonstrate that this platform can be used to administer nearly any macromolecule into virtually any cell type in a high throughput manner.[9]

Sharei *et al.*[9] illustrated that forcing cells through constricted microfluidic channels smaller than the cell diameter exerts a shear force on the cell that opens momentary membrane disruptions. Any target molecule in the buffer near these disruptions can then rapidly diffuse into the cell if smaller than the opening. They fabricated a simple device with a single inlet and outlet port joined by 45 parallel channels, each with identical constrictions in each channel. Solutions of cells and target molecules were pressurized through the device and the cells were collected at the output. The collected cells were next evaluated for the effective delivery of the target molecules into the cytosol. As shown in Fig. 3, the delivery efficiency improves with increasing speed of the cells through the device, longer constriction lengths, and increasing number of restrictions per channel. Importantly, the figure also demonstrates that the cells remain viable after going through the device at typical cell transport speeds. By demonstrating bidirectional transport of the target molecules, the authors provided evidence that the delivery mechanism was diffusion through the disrupted cell membrane and not endocytosis. Finally, target biomolecules, such as proteins and antibodies, remained functional after delivery, enabling promising applications in biorecognition and gene silencing.

Though HeLa cells were the primary cell type employed in this article, the authors also were able to deliver targets to embryonic stem cells, fibroblasts, dendritic cells, and murine white blood cells, all of which are difficult to transfect. Both 3 and 70 kDa dextran were used to illustrate the importance and influence of the physical device parameters, but delivery of anti-eGFP to GFP-expressing HeLa cells proved that the targets maintained their functions after insertion. Other payloads included Apolipoprotein B, plasmids, carbon nanotubes, quantum dots, antibodies and gold nanoparticles. Some of these samples are quite challenging to deliver using other transfection methods, and often require chemical modifications. Of particular interest is the delivery of transcription factors to primary cells. The authors delivered four different proteins to human fibroblast cells using both the constriction device and a standard cell-penetrating peptide (CPP) method. The CPP method trapped much of the proteins within endosomes, while the constriction device successfully delivered the factors throughout the cytosol and specifically to the cell nucleus. Additionally, NuFF cells were treated with the four transcription factors using the device, and the effectiveness of the strategy was evaluated with electroporation and CPP methods. The device was over 10× more efficient than the other methods and the resultant cells were able to express embryonic stem cell markers and differentiate into all three germ layer cell types.

Based on this extensive study, the authors have generated a design parameter guide for transfecting other cell types with this constriction device. Even without optimization, the current prototype can typically treat 20 000 cells s^{-1} with delivery efficiencies approaching 80% using the dextran–HeLa benchmark. The only minor weakness was device clogging over time, but reusability was not a focus of this work and nearly one million cells can be treated on a single device before failure. With the ability to deliver targets at high throughput and the flexibility in cell type and target selection, this technique outperforms the standard membrane poration or target modification methods. This versatile approach has the potential to revolutionize studies in drug discovery, therapeutics, cellular function and biomolecular sensing.

References

1 M. F. Ciaccio, J. P. Wagner, C.-P. Chuu, D. A. Lauffenburger and R. B. Jones, *Nat. Methods*, 2010, **7**, 148–155.
2 R. A. O'Neill, A. Bhamidipati, X. Bi, D. Deb-Basu, L. Cahill, J. Ferrante, E. Gentalen, M. Glazer, J. Gossett, K. Hacker, C. Kirby, J. Knittle, R. Loder, C. Mastroieni, M. MacLaren, T. Mills, U. Nguyen, N. Parker, A. Rice, D. Roach, D. Suich, D. Voehringer, K. Voss, J. Yang, T. Yang and P. B. Vander Horn, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 16153–16158.
3 A. J. Hughes, R. K. C. Lin, D. M. Peehl and A. E. Herr, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 5972–5977.
4 A. J. Hughes and A. E. Herr, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 21450–21455.
5 V. Lecault, M. VanInsberghe, S. Sekulovic, D. J. H. F. Knapp, S. Wohrer, W. Bowden, F. Viel, T. McLaughlin, A. Jarandehei, M. Miller, D. Falconnet, A. K. White, D. G. Kent, M. R. Copley, F. Taghipour, C. J. Eaves, R. K. Humphries, J. M. Piret and C. L. Hansen, *Nat. Methods*, 2011, **8**, 581–586.
6 I. Barbulovic-Nad, S. H. Au and A. R. Wheeler, *Lab Chip*, 2010, **10**, 1536–1542.
7 P. C. Gach, W. Xu, S. J. King, C. E. Sims, J. Bear and N. L. Allbritton, *Anal. Chem.*, 2012, **84**, 10614–10620.
8 J. Lee, A. Sharei, W. Y. Sim, A. Adamo, R. Langer, K. F. Jensen and M. G. Bawendi, *Nano Lett.*, 2012, **12**, 6322–6327.
9 A. Sharei, J. Zoldan, A. Adamo, W. Y. Sim, N. Cho, E. Jackson, S. Mao, S. Schneider, M.-J. Han, A. Lytton-Jean, P. A. Basto, S. Jhunjhunwala, J. Lee, D. A. Heller, J. W. Kang, G. C. Hartoularos, K.-S. Kim, D. G. Anderson, R. Langer and K. F. Jensen, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 2082–2087.

