MICROFLUIDIC DEVICE FOR IMMUNOBLOTTING

This application claims priority to U.S. provisional patent application 61/777,682, filed March 12, 2013, which is incorporated herein by reference in its entirety.

FIELD

The present invention provides microfluidic devices, systems, and methods for use in immunoblotting applications. In particular, devices and methods provided herein have the advantages of traditional Western blotting with increased throughput and multiplexability, and decreased time, sample, and reagent requirements.

BACKGROUND

Traditional Western blot analysis (or immunoblotting) is used to detect a specific protein in a cell, tissue, organ, body fluid, etc. The technique is a mainstay of biochemistry and molecular biology. Proteins in a sample are separated by gel electrophoresis (e.g., SDS-PAGE), and then transferred from the gel to a membrane (e.g., nitrocellulose or PVDF). The membrane is then stained with antibodies specific for a target protein. Although reliably yielding useful information, the technique is: time consuming, requiring of technical skill, reagent intensive, limited throughput, and poorly suited for multiplexing. What is needed is a technique that provides the advantages of traditional Western blotting with the increased throughput, multiplexability, and reduced reagent usage.

SUMMARY ~~OF THE INVENTION~~

The present disclosure~~invention~~ provides microfluidic devices, systems, and methods for use in immunoblotting applications. In particular, devices and methods provided herein have the advantages of traditional Western blotting with increased throughput and multiplexability, and decreased time, sample, and reagent requirements.

~~In some embodiments, t~~The present ~~invention~~ disclosure provides microfluidic immunoblotting devices comprising a flat membrane-contacting surface and a plurality of non-connected parallel microfluidic channels, wherein the length of said microfluidic channels are open to the membrane-contacting surface.

In an aspect, there is provided a microfluidic immunoblotting device comprising a flat membrane-contacting surface and a plurality of non-connected parallel microfluidic channels, wherein the entire length of said microfluidic channels are open to the membrane-contacting surface. In some embodiments, the microfluidic channels are spatially grouped into two or more

channel sets. In some embodiments, each of the two or more channel sets comprises 2-20 microfluidic channels. In some embodiments, the distance separating microfluidic channels with a channel set is less than the distance between channel sets. In some embodiments, a microfluidic channel (e.g., each of the microfluidic channels) comprises a reservoir at one end of the microfluidic channel. In some embodiments, a microfluidic channel (e.g., each of the microfluidic channels) comprises reservoirs at both ends of the microfluidic channel. In some embodiments, each of the microfluidic channels is 50-300 μm wide. In some embodiments, each of the microfluidic channels is 100-200 μm wide. In some embodiments, each of the microfluidic channels is 140-160 μm wide. In some embodiments, each of the microfluidic channels is 30-200 μm deep. In some embodiments, each of the microfluidic channels is 50-150 μm deep. In some embodiments, each of the microfluidic channels is 90-110 μm deep. In some embodiments, the ratio of width to depth of the microfluidic channels is less than 10:1 (e.g., <9:1, <8:1, <7:1, <6:1, <5:1, <4:1, <3:1, <2:1). In some embodiments, the ratio of width to depth of the microfluidic channels is between 3:1 and 1:3 (e.g., 2:1 to 1:2). In some embodiments, the ratio of width to depth of the microfluidic channels is 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:3, 1:4. 1:5, 1:6, 1:7, 1:8, or 1:9. In some embodiments, the ratio of width to depth of the microfluidic channels is between 3:1 and 1:3 (e.g., 2:1 to 1:2). In some embodiments, the device comprises polydimethylsiloxane (PDMS). In some embodiments, the device consists essentially of polydimethylsiloxane (PDMS). In ~~some embodiments, the present invention~~another aspect, there is provided~~s~~ a method~~s~~ of immunoblotting comprising applying antibody solutions to a membrane using a device ~~of the present invention~~as defined above.

~~In some embodiments, t~~The present disclosure~~invention~~ provides systems (e.g., assays) comprising a device ~~of the present invention~~as defined above and a membrane.

In another aspect, there is provided a system comprising a device as defined above and a membrane. In some embodiments, an activating solution is located or placed between said device and said membrane. In some embodiments, the activating solution comprises polysorbate-20 (Tween-20) and BSA. In some embodiments, the activating solution comprises 0.01-0.5% Tween-20 and 0.01-0.5% BSA. In some embodiments, the activating solution comprises 0.05-0.15% Tween-20 and 0.05-0.15% BSA. In some embodiments, the activating solution comprises 0.1% Tween-20 and 0.1% BSA. In some embodiments, the membrane comprises membrane nitrocellulose or polyvinylidene fluoride (PVDF). In some embodiments, the membrane comprises peptides, polypeptides, or proteins on or

within it. In some embodiments, systems further comprise one or more antibody-containing solutions within the microfluidic channels. In some embodiments, each microfluidic channel of a channel set comprises a different antibody-containing solution. In some embodiments, each channel set comprises the same combination of antibody-containing solutions. In some embodiments, each microfluidic channel comprises a different antibody-containing solution. In some embodiments, the present disclosure~~invention~~ provides a method~~s~~ of immunoblotting comprising applying antibody solutions to a membrane using system as defined above~~of the present invention~~.

~~In some embodiments, the present invention~~There is provide~~ds~~ a method~~s~~ of immunoblotting comprising: (a) transferring proteins from a sample onto a membrane; (b) placing a membrane-contacting face of a microfluidic immunoblotting device onto the membrane; (c) introducing (e.g., injecting) an activating buffer into microfluidic channels of said device; (d) introducing (e.g., injecting) primary antibody solutions into the microfluidic channels of said device; and (e) detecting binding of antibodies with said antibody solutions to said proteins.

In another aspect, there is provided a method of immunoblotting comprising: (a) transferring proteins from a sample onto a membrane; (b) placing a membrane-contacting face of a microfluidic immunoblotting device onto the membrane; (c) injecting an activating buffer into microfluidic channels of said device; (d) injecting primary antibody solutions into the microfluidic channels of said device; (e) detecting binding of antibodies with said antibody solutions to said proteins.

BREIF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic of a microfluidic immunoblotting device and an interface with a membrane. Three microfluidic channels overlie each sample lane that can be used to probe for three different proteins per lane.

Figure 2 shows antibody dilutions in clinical PBMC samples. (A) Traditional immunoblotting with primary antibody against RelA/p65 using 1:500 and 1:5000 antibody dilutions. (B) Microfluidic immunoblot probing for RelA/p65 using the same conditions as demonstrating similar signal intensities and the flexibility to do several dilutions on the same sample.

Figure 3 shows detection of STAT3 phosphorylation in response to inflammatory stimuli. (A) Traditional immunoblots of RAW264.7 cell lysates showing phosphorylation of STAT3 in response to LPS stimulation. (B) Microfluidic immunoblot on same RAW264.7

cell lysates as (A). The microfluidic device allows for simultaneous monitoring of phospho-STAT3 and STAT3 in the same sample without the need for stripping or reprobing of the PVDF membrane.

Figure 4 shows protein immunoblots of the MAPK pathway with two different chemiluminescent detection modalities. (A) Traditional immunoblots of RAW264.7 cell lysates with or without LPS for 45 minutes. Membranes were probed for