Miniaturization of Thin-Film Direct Coating Technology for New Biomedical Applications

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Abstract

In analytical chemistry, the consumption of the costly materials (e.g., antibody, enzyme etc.) and the tedious operation time conducted by traditional methods has been demonstrated to be significantly reduced by introducing the thin-film coating technology. Based on the basic principle of chemistry reaction, a novel thin-film direct coating (TDC) technique has been successfully developed and tested in the miniaturized desktop prototype of a thin-film precision coating system to profoundly improve the efficiency of western blotting (WB) method that is widely used in life science studies and clinical diagnoses. The suction function has been also added in the TDC system, denoted as TDCS, to further enhance its performance. By using this technique, a thin layer of antibody or buffer solutions could be easily and homogenously coated on the PVDF membrane within seconds and then incubated and washed by automatic control. The consumption amount of antibody could be reduced by a factor of up to 10^4 in comparison with that by the conventional WB. The operation time for completely finishing a high quality of WB can be reduced from 3 hr in conventional WB to about 5 min or even less. In addition, the signal-to-noise ratio of the immunoblotting by TDCS can be markedly increased. TDCS WB could particularly detect extrinsic glutathione-S-transferase (GST) proteins added in crude *Escherichia coli* or 293T cell lysates. Furthermore, the multi-channel TDCS WB has demonstrated to be a reliable method for parallel detections of different antigens with specific antibodies in a single experiment. The simple, material-saving and innovative new TDC system offers various potential applications in simultaneously and specifically finishing multiple antibody–antigen screenings in a fast and single experiment, demonstrating that it does perfectly mesh with the demands of the high reliability of bio-medical diagnoses.

Introduction

Western blotting (WB), which is comprised of various biochemical techniques, is widely used in life science studies and clinical diagnoses.[1] WB can identify specific targets in protein mixtures because of specific antibody–antigen interactions. However, several drawbacks, such as its high costs of excessive antibody consumption and time-intensive procedures still limit WB's applicability in bio-screening. In response to these

issues, microfluidic methods have been gradually employed to lower down the antibody consumption and system automation.[2-4] For instance, He and Herr[2-3] introduced a glass-based microfluidic chip, which was fabricated by the wet etching process, and a photo-patterned membrane for antibody-based in-gel blotting. The whole immunoblotting process only consumed ~1 μg of antibody, which was benefited from the scaling effect of microfluidic methods. However, it can detect only one target protein in each assay. Recently, a method incorporating microfluidics with a microtiter plate–based μWB array was developed to simultaneously analyze 10 proteins[4]. The μWB array improved the immunoassay, but it may require complex micromachining techniques.

In addition to develop a system that can reduce antibody consumption, the efficiency and sensitivity of WB from the perspectives of operation and incubation time has same economic values as antibody usage amount during the probing process. Recently, the SNAP i.d. 2.0 Protein Detection System (by Merck Millipore)[5] has significantly reduced the operation time from 3 hr to 30 min by using an active vacuum mechanism for conducting antibody probing and washing steps. Regretfully, in this system, only the durations in staining and washing steps are improved, whereas antibody consumption is not, and therefore a general application of this method is still very limited in the biochemistry laboratory. Recently, we've proposed a plane coating technique[6] to remarkably reduce antibody consumption in the labelling process of WB, namely thin film direct coating (TDC) WB method.[7] Conceptually, TDC WB has demonstrated that the antibody consumption can be further significantly reduced by shortening the coater width. This means that the newly developed TDC coater would produce a narrow line, instead of a plane, for the WB probing with much lower material consumption but without losing its sensitivity. In this study, the suction mechanism has been added in the TDC WB method to further shorten the operation time of the labelling process. It demonstrated that the TDC with suction (TDCS) technique can increase local antibody concentrations for antibody–antigen interaction, reduce the incubation and washing times, remove residual antibodies present on the polyvinylidene fluoride (PVDF) membrane, decrease the background noise, and consequently enhance detection sensitivity. It is highly expected that by applying TDCS in immunodetection, the functionality to simultaneously detect multiple proteins within a shorter processing time and the feasibility for parallel detections of different antigens with specific antibodies in a single experiment can be greatly enhanced in the general biochemistry laboratory.

Methodology

The schema of TDCS WB is illustrated in Figure 1. The coater can be silicon coater[6-7] or the capillary tube[8] for wet coating films of different coating width. Through the relative motion of the coating head and substrate, the user defined uniform antibody film can be homogeneously coated on the PVDF membrane. After the coating process has been completed, the PVDF membrane was incubated at room temperature for 2-10 min and ready for the subsequent three washing steps with suction. To enhance the kinetics efficiency between antibody-antigen, the desktop coating machine was built to achieve the suction function by connecting the suction chamber to a vacuum pump. Detailed descriptions can be found in Liu et al.[9]

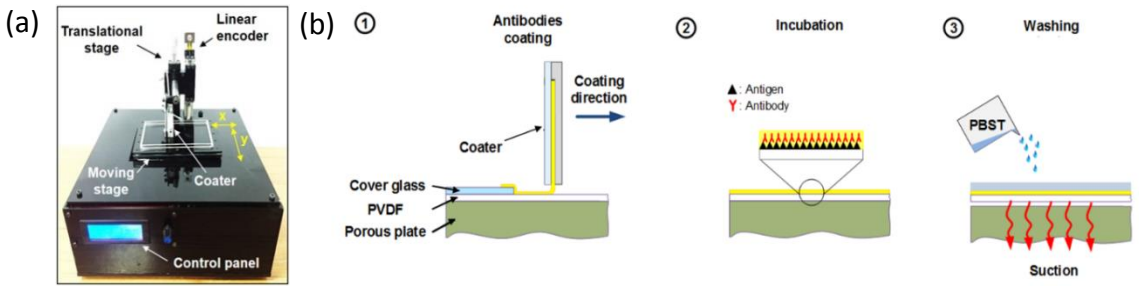

Figure 1. (a) The system photo and (b) process schema of TDCS WB[9].

Results and Discussions

TDCS WB has been successfully applied for WB and has considerably increased WB efficiency and reduced antibody consumption. The detection dynamic range has been tested in comparison with that of conventional WB as shown in Figure 2. The consumption of anti-GST antibody was 2 μg in conventional WB and was only 0.02 μg in TDCS WB. Furthermore, the amount can be even reduced to 10^{-4} μg by shortening the coater width to a 100 μm capillary tube with mixed BSA, GST, and Ub antibodies (Figure 2c). In addition, suction mechanism of TDCS WB also reduced the nonspecific blotting background. The lower limit of detection can be easily observed by extrapolating the linear fitting curve, and is much smaller than 90 ng. Table 1 shows the comparisons of operation time durations used in conventional WB and TDCS WB. It is worth noting that the coating speed of 1 mm/s tested in TDCS WB was very slow so that the duration the coating operation for TDCS WB could be almost negligible in the real application. This means that the operation time could be even less than 2 min (instead of 30 min in SNAP i.d. 2.0 Protein Detection System[5]).

Table 1. Comparisons of suggested operation time in Conv. WB and TDCS WB

Method	**Operation time duration (min)**				**Total**
	1st Ab	1st Wash	2nd Ab	2nd Wash	**(min)**
Conventional WB	60	30	60	30	180
TDCS WB	2	0.5	2	0.5	5

Conclusions

A novel, highly integrated desktop coating machine with suction functionality has been successfully employed to significantly reduce the antibody usage amount by 100-fold, and remarkably shorten the operation time in immunodetection. The highly efficient, user friendly, and easily adapted TDCS method can therefore consequently be expected to widely spread in general biochemistry laboratory.

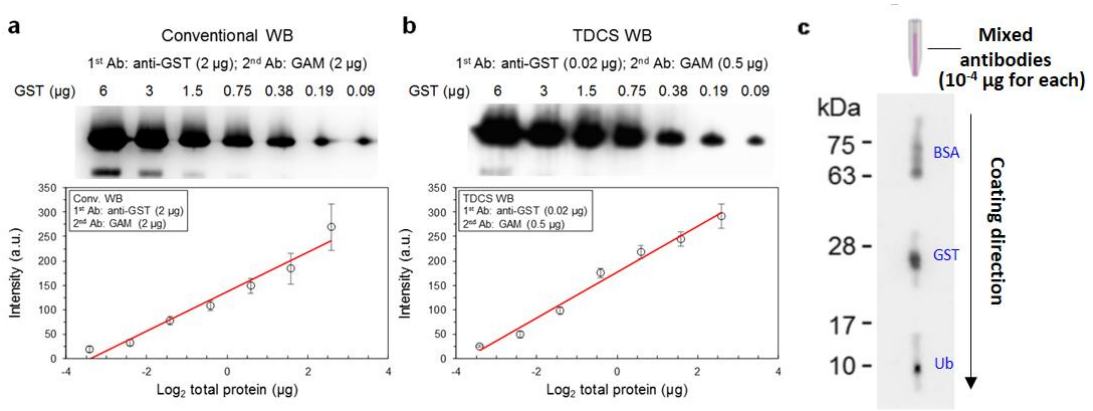

Figure 2. The detection dynamic range comparison in conventional WB and TDCS WB.

Acknowledgement

This research was supported by the Ministry of Science and Technology, ROC, under Grant No. MOST 104-2221-E-002-138-MY3 and MOST 104-2321-B-002-019 and the Industrial Technology Research Institute (ITRI), Taiwan, R.O.C. (contract no. 102-S-C19).

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