WASHING-FREE ALL-IN-ONE IMMUNOSTAINING REACTION OF MULTI-STEP QUANTUM DOT LABELING REAGENTS

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ABSTRACT

We firstly propose a novel immunostaining device which enables simultaneous reaction of multi-step labeling reagents in a microfluidic device. A microfluidic diffusion of the primary and quantum dot (QD)-labeled secondary antibodies (Abs) with a continuous flow in a microchannel induces sequential immunoreactions without additional washing steps, maintaining target antigen-binding sites of primary Abs. To ensure homogeneous dispersion of both primary Abs and QD-labeled secondary Abs on the protein coated surface, a T-shaped microchannel with slanted ridges on top of the channel was exploited to induce homogeneous dispersion of two Abs on the target protein coated region.

KEYWORDS

Microfluidics, Immunoassay, Immunocytochemistry, Quantum dot, Protein array

INTRODUCTION

Fluorescence-based immunoassays using quantum dot (QD) nanoparticles are used to measure a fluorescent color change of QD-labeled secondary antibodies (Abs) as the signal for the presence of analytes [1]. This indirect immunolabeling or immunostaining method generally requires several incubation and washing steps, in which each process still remains a laborious and time-consuming work (Figure 1A). Here, we present a new microfluidic immunostaining device which enables all-in-one reaction of multi-step labeling reagents simultaneously. A microfluidic diffusion in a continuous flow yields sequential immunoreactions of primary and QD-labeled secondary Abs on the immobilized proteins without washing steps, conserving antigen-binding sites of Abs. Figure 1B depicts a schematic illustration of this phenomenon, showing that the primary Abs and QD-labeled secondary Abs in a microchannel are sequentially conjugated to target proteins by flows along each streamline.

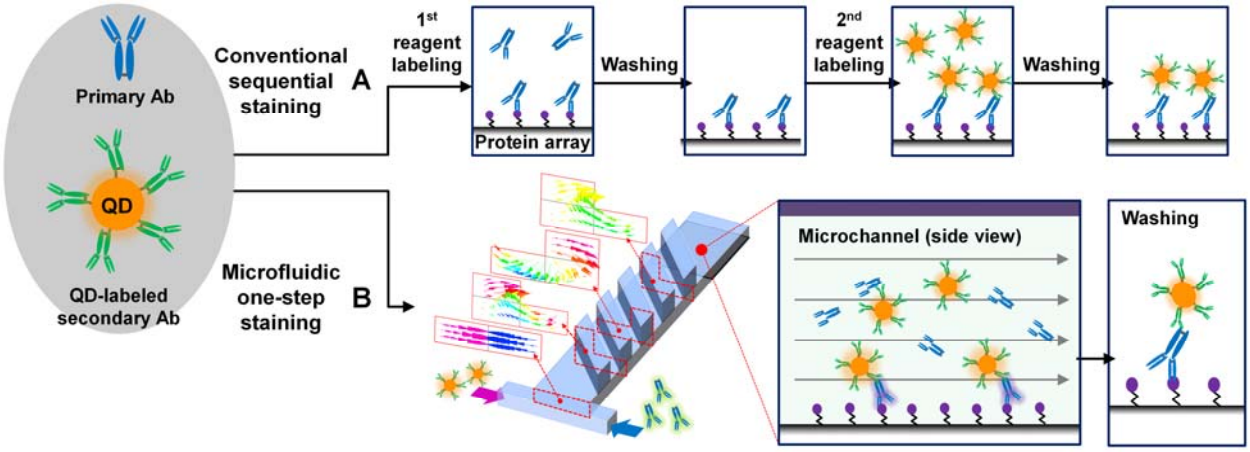

Figure 1. Comparison of immunoreactions between (A) a conventional sequential staining method with washing steps and (B) a microfluidic immunostaining method without washing steps. (A) After binding of primary Abs to target proteins, quantum dot (QD)-labeled secondary Abs are conjugated to primary Abs with several washing steps. (B) A steady flow in a microchannel maintains target antigen-binding sites of primary Abs, reducing the formation of undesired immunocomplexes. Accordingly, primary Abs and QD-labeled secondary Abs are sequentially conjugated to target proteins by flows along each streamline.

EXPERIMENTAL

To demonstrate a protein assay, carcinoembryonic antigen (CEA) protein, rabbit anti-CEA Ab, QD605 (QD with an emission peak of 605 nm)-labeled goat anti-rabbit Ab were used. CEA proteins were patterned on the aldehyde activated glass to make a protein array. Briefly, CEA proteins were dissolved in PBS (pH 7.4, 40% glycerol) and the proteins were immobilized on the aldehyde activated slide through microchannels. After 1 h incubation on the glass slide, microchannels were removed to wash out the unbound Abs. After the blocking process with PBS containing 3% BSA, rabbit anti-CEA and QD605-labeled secondary Abs were incubated sequentially.

The cell block preparation was described in our recent work [3]. SK-BR-3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU ml^{-1} penicillin, and 100 mg ml^{-1} streptomycin. To make cell blocks, the harvested cells were centrifuged. After the fixation process in

formalin, cells were suspended in agar. Paraffin embedding process was followed to produce cell blocks. The cell blocks were then sectioned with 4 µm thickness using microtome. The sections were then mounted and baked onto positively charged slides, and they were performed to dry for 1 h at room temperature, followed by 1 h in a convection incubator at 60°C.

For the microfluidic all-in-one staining, bottomless microfluidic channels were mounted on the protein patterned slides or cell block slides. A weight was given to the microfluidic device to avoid any leakage between the microfluidic channel and the cell block slides. Two inlets of the microchannels then connected with syringes, primary and QD-labeled secondary Abs were injected to each inlet using pumps.

RESULTS AND DISCUSSION

To compare whether a mixture of Ab reagents (primary Abs and QD-labeled secondary Abs) can be captured on the target proteins under static (no flow) conditions, immunoreactions were carried out in the form of a mixture of Ab reagents or by two-step sequential reactions of Abs with washing steps (control). Anti-carcinoembryonic antigen (CEA) Ab and QD605-labeled goat anti-rabbit Ab were mixed prior to incubation. In contrast with control data, no QD signals were detected after incubation of primary Abs and QD-labeled secondary Abs on the CEA-coated glass slide (Figure 2). This is because antigen binding sites of primary Abs were fully blocked with secondary Abs before the incubation of Ab mixtures on the CEA-coated glass slide. Meanwhile, a simple T-shaped microchannel used for the verification of the microfluidic immunostaining shows that target proteins were well conjugated with primary Abs and QD-labeled secondary Abs at the interface region of the microchannel (Figure 3). This means that even though two reagents were in the interface region, antigen binding sites of primary Abs were not fully blocked in the microfluidic continuous flow.

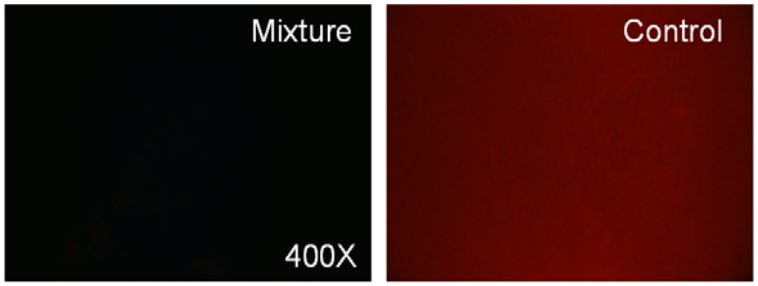

Figure 2. Fluorescent images of carcinoembryonic antigen (CEA)-coated slides after incubation of primary Abs and QD-labeled secondary Abs. Ab reactions were carried out in the form of a mixture of reagents (left) and by two-step sequential reactions with washing steps (right).

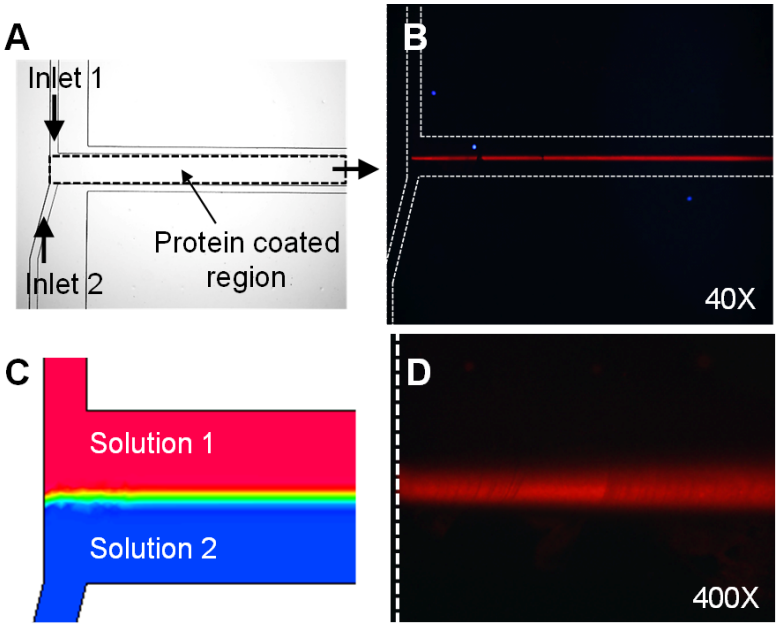

Figure 3. Ab mixture incubation with a T-shaped micromixer. Primary Abs were injected to the inlet 1 while QD-labeled secondary Abs were injected to the inlet 2. (A) A microchannel is mounted on the CEA protein coated glass slide. (B) Fluorescence image of Ab staining in a microchannel. The signal was only shown at the interface layer between two laminar flows. (C,D) QD stained area (D) was similar to the interface area in CFD simulation result (C) at the same condition.

To induce homogeneous dispersion of primary and QD-labeled secondary Abs on the target protein coated region, an efficient spiral flow should be induced in the microfluidic channel to the immunostaining channel design. In this paper, a T-shaped microchannel with slanted ridges on top of the channel was exploited to induce a spiral flow at low Reynolds number [2]. Figure 4 shows a schematic illustration of one-step immunostaining device and staining results with this system. To ensure homogeneous dispersion of both primary Abs and QD-labeled secondary Abs on the protein coated surface, a dispersion zone was prepared between inlets and reaction zone. QD probes were homogeneously labeled on the target protein-coated region on the protein microarray or the SK-BR-3 cell block. Human epidermal growth factor receptor 2 (HER2) on SK-BR-3 cells was labeled with HER2 Ab and QD605-labeled secondary Ab with this system. No non-specific binding of QD605 was found by observing the HER2 localization.

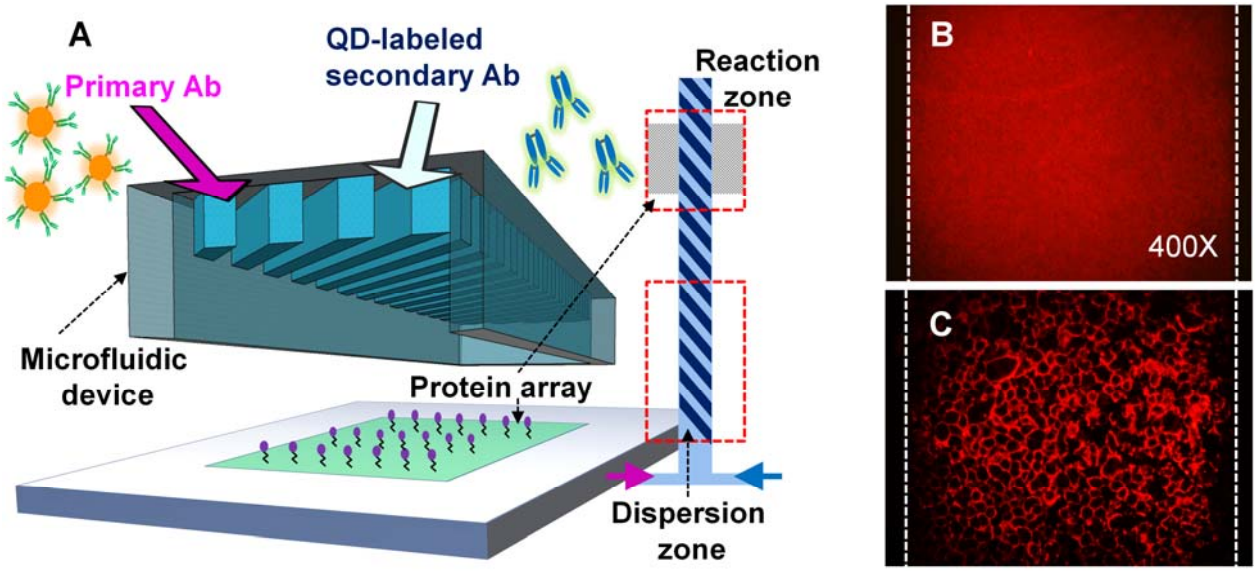

Figure 4. Schematic illustration of one-step immunostaining device and staining results. (A) Two Abs are flowed through the spiral flow inducing microstructures which induces an efficient dispersion of two Abs over the protein patterned regions under the microchannel. To ensure homogeneous dispersion of Ab mixture, the dispersion zone is located between inlets and the reaction zone. (B) A fluorescence image of CEA proteins stained with QD605 probes in a microchannel. (C) Immunocytochemistry was performed by using a section of SK-BR-3 cells mounted on a glass slide. The fluorescence signal was shown by human epidermal growth factor receptor 2 (HER2) Abs and QD-labeled secondary Abs. White dotted lines indicate the wall of microchannels.

CONCLUSION

In summary, we first proposed a novel immunostaining method which enables simultaneous reactions of multi-step labeling reagents in a microfluidic device. This one-step method will bring significant improvements in all kinds of immunoassays in terms of convenience, automation, and reduction of the total assay time.

ACKNOWLEDGEMENT

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