PROCEEDING

On-line microdevice for stress proteomics

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Abstract : The handing of the cells or tissues is essential for proteomics research or drug screening, where labor is not avoidable. The steps of cell wash, protein extraction, protein denaturing are complicated procedures in conventional method using centrifugation and pipetting in the laboratory. This is the bottle-neck for proteome research. To solve these problems, we propose to utilize the nanotechnology, which will improve the proteomics methodology. Utilizing the nanotechnology, we developed a novel microseparation system, where centrifugation and pipetting are needless. This system has a nanostructured microdevice, by which the cell handling, protein extraction, and antibody assay can be performed. Since cell transfer is needless, all cells are corrected without any loss during the cell-pretreatment procedures, which allowed high reproducibility and enabled the detection of low amount of protein expression. Utilizing the microdevice, we analyzed the stress induced proteins. We further succeeded the screening of food that was useful for immunity and found that an extraction from seaweed promoted the apoptosis of T-lymphoblastic cells. Here, we present an on-line microdevice for stress proteomics. J. Med. Invest. 52 Suppl. : 225-227, November, 2005

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INTRODUCTION

Protein expression profiling is necessary for studying cellular mechanism. For the purpose, 2-D and SDS - PAGE are usually utilized, which are powerful tools and can separate and analyze almost all cellular proteins. However, these are labor intensive and time consuming. In addition, relatively large amount of samples and reagents are requiring. To analyze the low amount protein expressions such as early stage of diseases, down sizing of analytical instruments is necessary.

Recently, microchip electrophoresis has been attracted much attentions as a promise tool for proteomics research and clinical diagnosis because it can reduce the sample scale. Protein sizing separation by microchip electrophoresis and an integrated microfabricated cell sorter system has been developed (1, 2), and these devices dramatically shorten the

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separation times. However, the sensitivity and separation ability are somewhat unsatisfactory. To improve the ability of microchip electrophoresis, we have developed several methods for microchip electrophoresis (3-10). Pressurization technique for microchip electrophoresis (3) , triple injection method (4) , reverse injection method using dynamic SDS coating (5) , improved sensitivity without denaturing (6) , polysaccharide or metylcellulose additive to the buffer as the dynamic coating (7) , new coating materials (8) , nanoball technique (9) , and nanosphere mixed medium(10) , etc. have been as unique techniques for high sensitivity and high-resolution separations. However, there are still many labor-intensive sample pretreatment procedures. Even if electrophoretic separation time is decreased, the total times such as collecting cells from cell culture, cell wash, changing media, several stimulations for drug discovery, antibody analysis, and protein extraction from cells, will not be reduced.

To avoid these time-consuming steps in the analysis of cell-free extracts, an on-chip single-cell expression analysis system (11) as well as an on-chip immunoassay system (12-20) , a system for single-cell level direct observation by microscope (21, 22) and an on-chip microculture system (23) have been developed. In the on-chip single cell analysis system, cell culture, chemical stimulation, and detection are integrated on a microchip. However, these techniques sometimes require a robust microchip, high-strict techniques, or strict flow control using an external pump. These can be difficult to use for disposal chip for clinical diagnosis or drug screening for high-throughput screening (HTS) systems. Simpler and easier cell pretreatment systems are required.

In this paper, we review a novel cell culture and cell pretreatment system that integrated all pretreatment steps and that requires only a microchip device, which lacks pipetting and centrifugation steps. This system was utilized to analyze protein expression during stress stimulations and found that a seaweed extraction promotes apoptosis.

MICRODEVICE

Two types of integrated cell culture microdevices (hand-made by Nisshin Medical Instrument, Tokushima, Japan) were fabricated. A hand-made microdevice (Nisshin Medical Instrument ; acrylic plate : 45×65 mm, 2-3 mm thickness (14-21 μL) ; well : 96 holes, 3 mm diameter ; bottom : 28 μm plastic sheet (Clear Seal, Nippon Genetics, Tokyo, Japan) covered at the bottom by heating). Another chip (acrylic plate 60×80 mm, 2-4 mm thickness) was drilled with 48-96 holes (5-6.5 mm diameter ; total volume of 40-130 μL). An～1 mm thick acrylic plate was covered at the bottom by adherence. Former can be connected to an electroseparation chip (i-chip 12 made of poly (methyl methacrylate) , Hitachi Chemical, Hitachi, Japan). The inner cup (8-well strip; Nalge Nunc International, New York) was re-formed with a 3-8 micron pore membrane (Nalge Nunc International) for the former microdevice. The inner cup was inserted into the microdevice well. The former connected type of microdevice is used for the SV 1200 of the Hitachi system. The latter, separated type of microdevice is used for the Agilent system (Agilent Technologies, Waldbronn, Germany) for preparation chips. For the ‘‘conventional’’ method, a conventional centrifugation tube (15mL) , microcentrifuge tube (1.5mL) , and culture dish (35mm) were used.

FOR STRESS PROTEOMICS

Utilizing this technique, we attained the electrophoretic separation of proteins extracted from cells within 1 min per 12 samples and we could readily screen the drug or food. We found that seaweed extraction promotes the apoptosis induction of T-lymphoblastic cells. Our novel pretreatment microdevice enables the easy analysis of extracted proteins without sample loss during the procedure, and also allows development of HTS systems of proteome analysis using cells. This is especially important for the analysis of small amounts of marker proteins, which are early stage of diseases.

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