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Self-Contained On-Chip Cell Culture and Pretreatment System

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Abstract: In this study, we describe a simple on-chip cell culture and pretreatment system that requires no external machines. Conventional cell culture utilizes culture dishes or microtiter plates, where pipetting and centrifugation are indispensable for washing cells and changing media. However, our microdevice requires no external centrifugation or pump. Utilizing this microdevice, we attained dramatically shorter total analytical time with a high-throughput screening system for proteomic analysis (1 min per 12 samples; one eightieth of the conventional time). Protein expression of Jurkat cells during stress-shock induced apoptosis was readily analyzed using this system. We found that a seaweed extraction effectively induced apoptosis of Jurkat cells.

Keywords: cell culture • microchip • high-throughput screening systems • apoptosis

Introduction

Protein expression profiling is necessary for studying cellular mechanisms. Conventional 2-D gels, SDS-PAGE and mass spectrometry (MS) are powerful tools that separate and analyze almost all cellular proteins, but they are labor intensive and time-consuming. Another powerful tool for proteomics is the protein antibody microarray system. Recently, protein sizing separation by microchip electrophoresis (μ-CE)[1] and an integrated microfabricated cell sorter system have been developed,[2] and these devices dramatically shorten the separation time. However, there are still many labor-intensive sample pretreatment procedures. Even if the electrophoretic separation time is decreased, the total time for harvesting cells from cell culture, changing media, stimulation, and sample pretreatment takes several hours. Some available assay kits have been already provided, which are utilized for only restricted assays. So we often rely on the conventional cell culture system that utilizes culture dishes or microtiter plates, where pipetting and centrifugation procedures are sometimes necessary for washing cells, exchanging media, and extracting proteins.

To avoid these time-consuming steps in the analysis of cell-free extracts, an on-chip single-cell expression analysis system,[3,4] as well as an on-chip immunoassay system,[5−14] a system for single-cell level direct observation by microscope,[15,16] and an on-chip microculture system[17,18] have been developed. In the on-chip single cell analysis system, cell culture, chemical stimulation, and detection are integrated on a microchip. Nevertheless, 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 materialization in a high-throughput screening (HTS) systems for proteomic analysis and medical diagnoses. Simpler and easier cell culture systems are required, especially for long-term (24−48 h) cell culture and sample pretreatment.

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

Experimental Procedures

Microdevice. Two types of integrated cell culture microdevices (handmade by Nisshin Medical Instrument, Tokushima, Japan) and our novel cell culture system are illustrated in Figure 1. Our 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). A ∼1-mm thick acrylic plate was covered at the bottom by adherence (Figure 1A). Another handmade 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) connected to an electroseparation chip (*i*-chip12 made of poly(methyl methacrylate), Hitachi Chemical, Hitachi, Japan) was also used (Figure 1B). The inner cup (8-well strip; Nalge Nunc International, New York) was re-formed with a 3−8 μm pore-polycarbonate membrane (Nalge Nunc International) for the former microdevice. Another handmade 12-well inner cup that incorporated wire and was positioned above a membrane was made for the latter microdevice. The inner cup was inserted into the microdevice well (Figure 1A,C). Approximately 20−75 μL of culture volume was then added. The appropriate well size was selected according to the experimental purpose. The former, separated type of microdevice is used for the Agilent system and in other commercial electrophoresis separation chips. The latter connected type of microdevice is used for the SV1200 of the Hitachi system. A thermoplate (Tokai Hit, Fujinomiya, Japan) controlled the temperature. For the "control" method,

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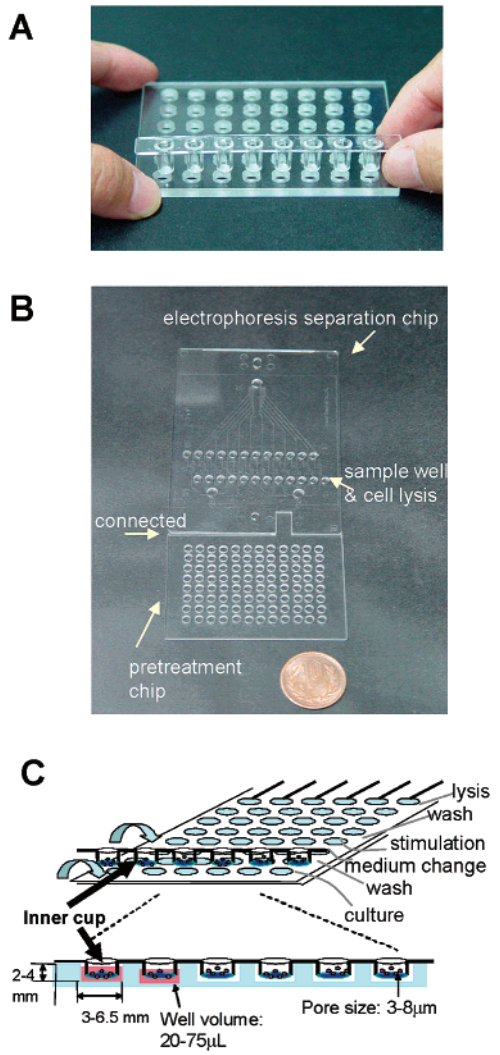

Figure 1. Integrated cell culture microdevices. (A) Photograph of the microdevice (handmade 48-well microchip (Nisshin Medical Instrument) and 8-well inner cup) that integrates cell culture, washing cells, stimulation, lysis, and immunoassays. (B) Photograph of the integrated cell culture microdevice (handmade 96-well microchip by Nisshin Medical Instrument) connected with an electrophoresis separation chip (i-chip12; Hitachi Chemical). (C) Our proposed cell culture system on the microdevice. The cells are placed into the inner cup and inserted into wells that are filled with culture medium. During the media exchange, washing, and cell lysis, the inserted cups containing cells are moved into the next row of wells, which are filled with new buffer or reagent.

a conventional centrifugation tube (15 mL), microcentrifuge tube (1.5 mL), and culture dish (35 mm) were used.

Samples and Reagents. Human Jurkat cells, which are derived from a T lymphoblastic cell line (Dainippon Pharm, Suita, Japan), were cultured in RPMI 1640 medium (Nissui, Tokyo, Japan) with 10% fetal bovine serum (FBS, Equitech-Bio, Kerrville, TX). Next, the medium was changed to an FBS-free buffer and cells were cultured at 37−51 °C for 30 min. The cultured Jurkat cells were lysed in cellytic-M (Sigma-Aldrich, Tokyo, Japan) for 5 min. For the "microdevice" sample, all cell culture procedures were performed on the microdevice. The concentrations of the extracted proteins were determined by a spectrophotometer (ND-1000; Nano Drop Technologies) using the BCA protein assay kit and standard BSA (Pierce, Rockford). The extracted protein samples were analyzed without being concentrated. Rabbit anti-active caspase-3mAb (BD Bioscience, San Jose), Cy5-conjugated AffiniPure F(ab′)Fragment Goat anti-rabbit IgG(H+L) (Jackson Immuno Research, West Grove), and BD FACS permeabilizing solution 2 (BD Bioscience) were used for the caspase 3 assay. The Annexin V-biotin apoptosis detection kit (Oncogene Research Products, San Diego), and Fluorolink Cy5 labeled streptavidin (Amersham Pharmacia Biotech, Buckinghamshire, England) were used for the annexin V apoptosis assay. A staining buffer (PBS including BSA and NaN_2 0.05%) was also used. Camptothecin (Sigma-Aldrich) was the apoptosis-inducing drug. A pure β-1,3-glucan (Wako Pure Chemical Industries, Japan) and seaweed extractions were used as stress stimulators. The seaweed extraction was prepared from food seaweed (Wakame). After homogenizing the seaweed by stery mill (SM-1 Labcat, USA), the water soluble components were utilized.

***μ*-CE.** Microchips in Protein Chips (Protein 200 Plus Labchip; Agilent Technologies, Waldbronn, Germany), made from soda lime glass, were used for protein detection. The sieving matrix (Agilent Technologies), destaining buffer (Agilent Technologies), ladder (Agilent Technologies), and protein samples were loaded according to the manufacturer's instructions. Protein separation and detection were performed in an Agilent 2100 Bioanalyzer instrument (Agilent Technologies), with detection set at 630-nm emission. Another μ-CE system (cosmo-*i*; SV1210, Hitachi Electronics Engineering, Hitachi, Japan), equipped with a diode laser emitting at 635-nm was also used for the latter microdevice (Figure 1B). For this microdevice, a previously reported protein separation method was adopted.[19] All proteins were analyzed without denaturing, using a method that we developed previously.[20] The samples were injected via pressure (8.5 kPa for 1 s) on the loading channel, instead of by electrokinetic sample injection. Pressure was continuously applied to the separation channel in the absence of any electric potential, and samples were then separated at 1300 V.

Caspase 3 Assay. The caspase 3 assay consisted of two antibody reaction steps. Jurkat cells ($(1-5) \times 10^5$/mL; depended on the experiment) were treated with 6 μM camptothecin for 4 h or 37−51 °C (heat-shocked) for 30 min to induce apoptosis. Before heat shock, the culture medium was changed to FBS-free buffer. The control and treated cells were harvested, resuspended in BD permeabilizing solution, and incubated for 10 min at room temperature. After washing with staining buffer, cells were incubated with anti-caspase-3 antibody (5 μg/mL per 5×10^5 cells/100 μL) for 20 min at 0 °C. After washing with staining buffer, cells were incubated with the secondary antibody (5 μg/mL per 100 μL staining buffer) for 30 min at 0 °C. The reaction time, reaction volume, and antibody concentrations were optimized for the microdevice.

Annexin V Assay. Annexin V detects the phosphatidyl serine that appears at the surface of the plasma membrane during the early phase of apoptosis. Phosphatidyl serine, which is actively confined to the inner leaflet of the lipid bilayer in healthy cells, translocates to the outer layer, becoming exposed to the external cell surface. Annexin V belongs to a family of calcium and phospholipids−binding proteins with high affinity for phosphatidyl serine. It can be used as a sensitive probe for phosphatidyl serine exposure on the cell membrane surface. This assay was utilized to investigate the early stage of apoptosis. After treatment with camptothecin or heat-shock, cells treated with the kit, including a media binding reagent (10 μL

per 5 × 10^5 cells/500 μL) and annexin V-biotin (1.25 μL per 5 × 10^5 cells/500 μL), were incubated for 15 min at room temperature. The medium was changed to binding buffer, and 1 μL of 1 mg/mL Cy5-streptavidin was added. The solution was incubated in the dark (covered with tin foil) for 10 min at room temperature. Cells were washed once with binding buffer, then resuspended in water and analyzed. For the microdevice, the reaction time and reaction volume were optimized.

Results and Discussion

Proposed Microdevice for Cell Culture and Pretreatment. Multi-well parallel batch techniques are the most commonly applied techniques in HTS. Microdevices (Figure 1A (separated type) and 1B (connected type)) can substitute for the 96-well microtiter plate in a HTS system. The cell culture system on the microdevice is illustrated in Figure 1C. The first row of wells is filled with culture medium. Next, the cells are placed into the inner cup and inserted into the wells. During long-term culture (~48 h), the plate is covered with a gas permeable adhesive seal to avoid contamination and evaporation, and placed in a 5% CO_2-incubator. During the media exchange, the inserted cups containing the cells are moved into the second row of wells, which are filled with new buffer. Cells can be quickly transferred into new buffer without any pipetting and centrifugation. Stimulation, washing, and cell lysis can be performed in a similar manner. For short-term incubations (~2 h), such as for heat shock (37−51 °C) or stimulation, the microdevice temperature can be controlled by the thermoplate. The reagent for cell lysis (10−14 μL) is put into the last wells, in which the inner cups with cells are placed. Soluble protein can move into the well (out of the insert cup) through the membrane, and the inner cup containing the precipitate can be removed. Protein separation electropherograms with and without the inner cup membrane were compared. The same data were seen in both sample sets, indicating that there was little protein adsorption onto the inner cup membrane. In sum, this microdevice enables tissue culture tasks to be handled rapidly by eliminating the labor-intensive pipetting and centrifugation steps.

Figure 2A compares electropherograms, generated by the Agilent system, showing proteins extracted from Jurkat cells using the separated type of microdevice (Figure 1A) and a routine method using culture dishes and 15 mL centrifuge tubes. Prior to this operation, the cell density was adjusted for each group as described on the left side of each electrophoresis record. The protein analysis without denaturing method, which we previous developed, was adopted. The concentrations of total extracted proteins after pretreatment are indicated on the right side of each electropherogram. Both groups of electropherograms were similar; however, higher intensity peaks and lower levels of detection were obtained with the microdevice (left side of Figure 2Aa). This apparently was because the total number of proteins obtained by the microdevice was higher than that obtained using the routine method.

Table 1 (upper) compares the cell densities of Jurkat cells obtained using the microdevice (Figure 1A) and a conventional method after sample pretreatment (washing cells, changing media, etc.). A higher cell density was obtained with the microdevice, since there was minimal, if any, cell loss at each cell density. This contrasts with conventional methods, in which cell density is reduced by pipetting, decantation, or vacuuming of the supernatant, and by transferring cells from dishes to tubes (lower section of Table 1). The microdevice can also reduce standard errors between experimental runs, relative to the routine method. The relative standard deviation (RSD) values in 72 assays were <4% for the microdevice and 15−20% for the routine method (Table 1). Thus, the cell culture,

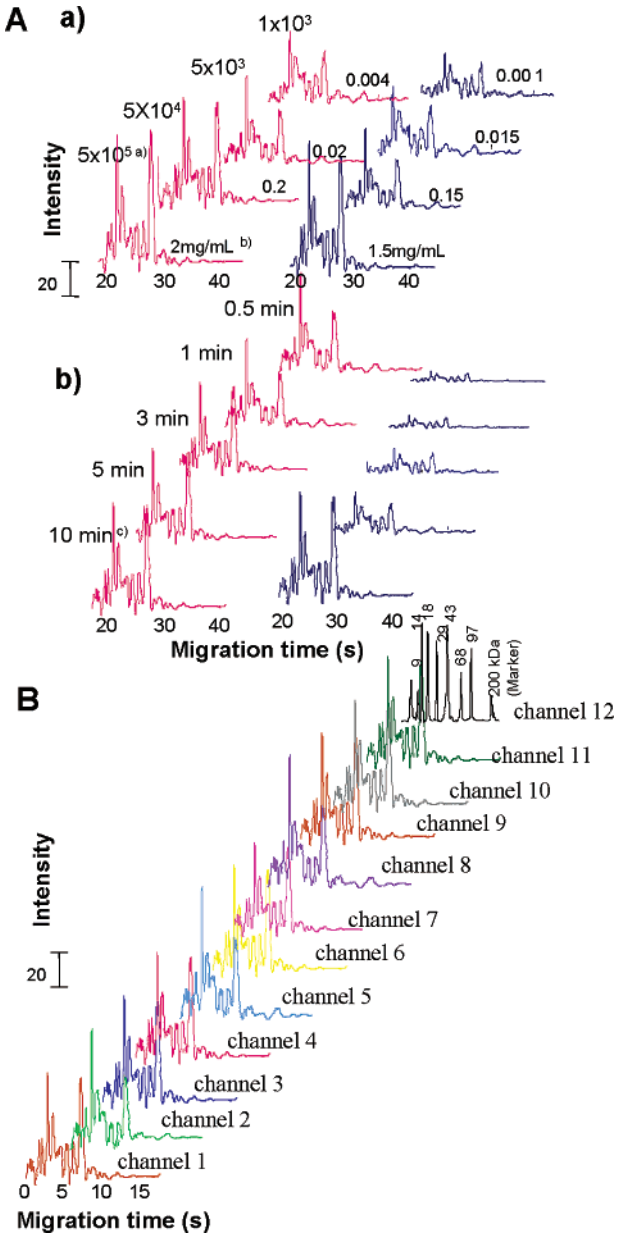

Figure 2. Electropherograms of proteins extracted from Jurkat cells. (A) (a) Comparison of peak intensity derived from initial cell density between the microdevice (Figure 1A) and a conventional method that utilizes culture dishes and centrifuge tubes. (b) Comparison of peak intensity derived from reaction times of the microdevice (Figure 1A) and by a conventional method. Protein separations were performed by the Agilent 2100 Bioanalyzer and Protein 200 Plus kit. (a) Initial cell density. (b) Total protein concentration obtained after several procedures. (c) Reaction time of cell lysis. (B) Electropherograms of 12 samples (11 sample from Jurkat cells and a protein ladder) using the connected type of microdevice (Figure 1B). The protein separations were performed according to an earlier method[19] using an SV1210 (Hitachi Electronics Engineering). Similar cell densities (5 × 10^4) of Jurkat cells were placed at channels 1−11, and a standard protein mixture (ladder) was placed in the 12th channel. Cell lysate (10 μL) was used for protein extraction from the cells.

Table 1. Cell Density Reduction after Procedures

	microdevice[b]		conventional[c]	
initial[a]	mean	RSD (%)	mean	RSD (%)
5.0	4.91	3.6	4.01	16.3
1.0	0.99	3.1	0.77	15.0
0.5	0.49	3.2	0.39	17.9
	(cell density ($\times 10^5$ cells/mL))			

	new[b]		conventional[c]	
procedures[d]	mean	RSD (%)	mean	RSD (%)
initial[e]	5.00		5.00	
first wash[f]	4.99	1.8	4.53	19.5
second wash[g]	4.96	3.6	4.22	17.0
third wash[h]	4.95	3.4	4.18	14.0
forth wash[i]	4.90	3.5	3.95	18.3
	(cell density ($\times 10^3$ cells/mL))			

[a] Cell density reduction (during pretreatment for Figure 2) depen ds on initial cell density. Initial cell density was adjust to 5, 1, or 0.5 $\times$ 10^5 cells/mL. [b] Microdevice (Figure 1A), $n = 72$; 8 samples/chip $\times$ 3 chips/day $\times$ 3 days. [c] 35 mm dish and 15 mL centrifuge tube, $n = 72$; each 8 dishes $\times$ 3 days. [d] Cell density reduction during each step. [e] Initial cell density; $\times 10^3$ cells/mL. [f] After culture. [g] After medium change. [h] After primary culture or stimulation. [i] After medium change to wash buffer.

wash, and treatment using the microdevice resulted in maintenance of cell density throughout the experiment.

We further investigated the time required for protein extraction from Jurkat cells (Figure 2Ab). Prior to the operation, cell density was adjusted to 5 $\times$ 10^4 cells for use in the microdevice (Figure 1A) and 7 $\times$ 10^4 cells for use in the conventional method. These values were estimated to be sufficiently intense, by referral to the data shown in Figure 2Aa. Through decreasing the reaction time, the conventional method produced reduced peak intensities, whereas the microdevice retained peak intensities between 30 s and 10 min. Only 30 s of lysis reaction time was necessary to carry out protein analysis from 5 $\times$ 10^4 cells using the microdevice.

By connecting all procedures to an electrophoretic separation chip (Figure 1B), cell culture, washing of cells, stimulation, protein extraction, and electrophoretic separation processes could be integrated. This microdevice can manipulate twelve samples simultaneously. The same cell density (5 $\times$ 10^4 cells) of Jurkat cells was introduced into each well in the first row of the microdevice (12 wells). The final step of cell lysis was carried out in the sample wells of the connected electrophoresis separation chip (Figure 1B). The extraction reagent (10 μL) was placed in the sample wells after buffer was injected in all channels. The inner cup including the cells was transferred to these wells and placed inside them. After 30 s, which is the minimum reaction time shown in Figure 2Ab, the inner cup was removed. Pipet transferring was unnecessary in this system. In addition, we adopted a previously developed sample injection method, the pressurization technique,[19] which can readily introduce the sample to the electrophoresis separation channel. This method enables twelve samples to be separated within 15 s. We further adapted the protein analysis method without denaturing[20] to this application as well. Twelve channels can be separated simultaneously in this system. Eleven wells (one channel was used for a standard protein separation) contained similar levels of proteins extracted from these samples (Figure 2B, RSD of 11 samples <5%), indicating that this system is well contained, with little sample loss. These data indicate that this microdevice enables proteins extracted from cells to be easily analyzed, without fear of losing precious samples during the process. All procedures, from the primary culture to the electrophoresis separation step, could be integrated in a microchip.

Table 2 (upper) summarizes the scaled down analysis time, cell density, and reagents required by the connected type of microdevice and an SV1200 system. The optimal conditions (5 $\times$ 10^4 of cell density, 10 μL of cell lysis reagent and 30 s of reaction time for the microdevice) were deduced from Figure 2A. The conventional method and Agilent system were used as controls. The microdevice could reduce the total analysis time, including cell pretreatments, to one minute, whereas the conventional method required 80 min for the analyses of 12 samples.

Apoptosis Assay Using the Microdevice. Cellular responses to stress signals, especially stress shock, have been studied extensively. Studies of the expression of stress shock proteins and their functional regulation are an important field in cell biology.[21] Protein expression during heat shock of Jurkat cells has been well characterized.[22−25] We already found that patterns of extraction proteins from heat-shock were differ from those from nontreated Jurkat cells.[19,20] In this time, the relation of heat-shock stress to Jurkat cells and apoptosis induction was investigated using the connected type of microdevice (Figure 1B).

Recently, β-1,3 glucan has gained much attention as an antitumor activator. We recently found that the effect of some strains of seaweed have the promoter for immuno regulation and some skin diseases.[26] The seaweeds include the component of β-1,3 glucan; however, the effective component is thought to differ and the mechanism of it is unknown. Seaweed extractions were also utilized as one of the stress shock factors applied to Jurkat cells during our developed system and the apoptosis observations were analyzed.

Figure 3 shows the results of electropherograms of the extracted proteins from stress-shocked Jurkat cells. The connected type of device (Figure 1B) and 12-channnel type of Hitachi microchip electrophoresis system were utilized for this screening. The electropherogram of stress shocked condition by a 0.01% seaweed extraction showed the same pattern as that of 6 μM camptothecin, which were different from those of subjected to heat-shock conditions and a pure 0.01% β-1,3 glucan. This pattern also differed from that obtained from heat shock conditions.[19,20] Camptothecin is widely known as a promoter of apoptosis induction. These data indicate that the seaweed extraction may be also a promoter of apoptosis induction.

Figure 4A summarizes the results of caspase 3 and phosphatidyl serine induction by apoptosis that occurred during stress-treatment of Jurkat cells. Figure 4Aa indicates that caspase 3 induction is dependent on heat-shock stress. Treatment with higher heat produced higher levels of caspase 3 induction. The caspase 3 results revealed that apoptosis induction could be detected in heat-shocked Jurkat cells. The seaweed extraction also showed caspase 3 induction. However, we failed to detect cell fragmentation and degradation in heat-treated cells by microscopic observation (Figure 4Ba). In contrast, the camptothecin-treated cells and 0.01% seaweed extraction, which had intense caspase 3 production, showed cell fragmentation owing to natural fragmentation (Figure 4Bb and c). The β-1,3 glucan failed to show the caspase 3 induction. Apoptosis inducing proteins were further analyzed by the annexin V assay (phosphatidyl serine detection), which indi-

Table 2. Scale down Using Microdevice

procedure	minimum requirement/ reaction microdevice[c]	volume/analysis time conventional[d]
	for pretreatment	
1. wash	2 s	10 min
2. medium change	1 s	5 min
3. lysis	30 s	20 min
4. denaturing	0 s	10 min
total[a]	33 s	45 min
total analysis time b	1 min	80 min
cell density (cells/mL)	5×10^4	1×10^5
minimum cell density (cells/mL)	1×10^3	5×10^4
	for immunoassay	
caspase 3 assay		
1. wash	1 s	10 min
2. BD permeabilizing solution	75 μL/5 min	500 μL/10 min
3. Anti caspase-3 antibody/staining buffer	0.1 μg/20 μL/5min	0.5 μg/100 μL/20 min
4. wash (staining buffer)	1 s	10 mim
5. secondary antibody/staining buffer	0.1 μg/20 μL/10 min	0.5 μg/100 μL/30 min
6. wash	1 s	10 min
total[a]	20.5 min	90 min
total analysis time[b]	21 min	120 min
cell density (cells/mL)	5×10^4	1×10^5
minimum cell density (cells/mL)	1×10^3	5×10^5

[a] Including or [b]excluding electrophoresis separation. [c] Using the microchip (Figure 1B) and our previous system19 using SV1200 system. [d] Using the conventional method and Agilent protein assay system. The values recommended in manufactures' instruction are adopted for conventional.

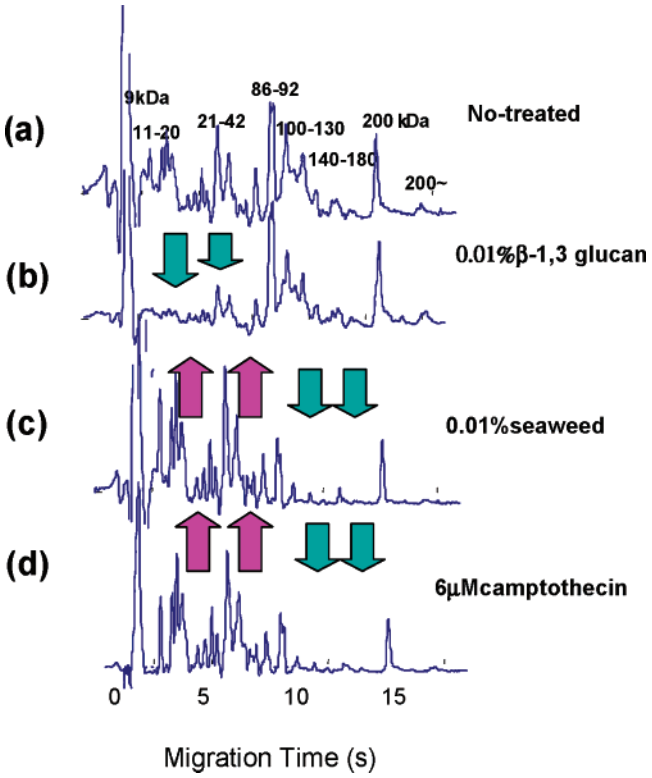

Figure 3. Electropherograms of stress induced proteins. (a) no-treated, (b) 0.01% β-1,3 glucan-treated, (c) 0.01% seaweed extraction-treated, and (d) 6 μM canptothecin-treated Jurkat cells. Each treatment was done for 30 min. Other conditions were identical to those in Figure 2b. Protein markers (9 kDa and 200 kDa) were used.

cates the first stage of apoptosis (Figure 4Ab). Phosphatidyl serine was also detected in the heat-treated Jurkat cells. The intensity depended on the heat treatment, indicating that the first stage of apoptosis was also induced by heat-shock. The progress of apoptosis seemed to be moderate for heat-shocked Jurkat cells. The 0.01% seaweed extraction also showed full intense phosphatidyl serine. In contrast, the pure β-1,3 glucan showed no production of phosphatidyl serine. These data indicate that the mechanism by 0.01% seaweed is different from those by heat-stress or β-1,3 glucan and 0.01% seaweed can directly introduce apoptosis, which seems similar to camptothecin treatment, but different from heat-treatment. It is known that seaweed extraction has the effect of skin diseases.[26] which may be related to apoptosis induction. More details are needed to resolve this mechanism. In this way, our microdevice can easily be utilized for screening for proteome research.

Table 2 (lower) compares the limits of required cell density, reaction volume, and total analysis time between the microdevice (Figure 1B) and a routine method (microtubes) during the immunoassay. Figure 4Ca shows optimization of the minimum reaction time for the caspase 3 assay pretreatment procedures for the microdevice. Similarly, the minimum reaction volumes for each process are shown (Figure 4Cb). Also, minimum antibody concentrations were determined. These data revealed that the microdevice could reduce the total analytical time and the reaction scale. The reaction volume was reduced about 5 times and the total reaction time was reduced about 6 times by the microdevice.

Comparison with Other Microchips. Western blotting, which is sometimes utilized for confirming a particular protein after analysis by 2-D, SDS-PAGE, μ-CE, or antibody microarrays, is a powerful technique to analyze a particular protein; however, these procedures are complicated and time-consuming. The on-chip cell analytical system[3−7,9,13] has integrated cell culture, chemical stimulation, and detection in a single microchip. An extended culture system is sometimes necessary, especially for time-course studies, such as DNA synthesis during the division cycle. In such studies, media exchange from FBS buffer to FBS-free buffer is required. Our microdevice can readily wash cells and exchange the medium. Recently, an on-chip microculture system[17,18] was developed. However, its construction appeared to be difficult to implement for high-throughput medical diagnoses. In the on-chip microculture system, a single cell or a relatively low cell density is cultured.

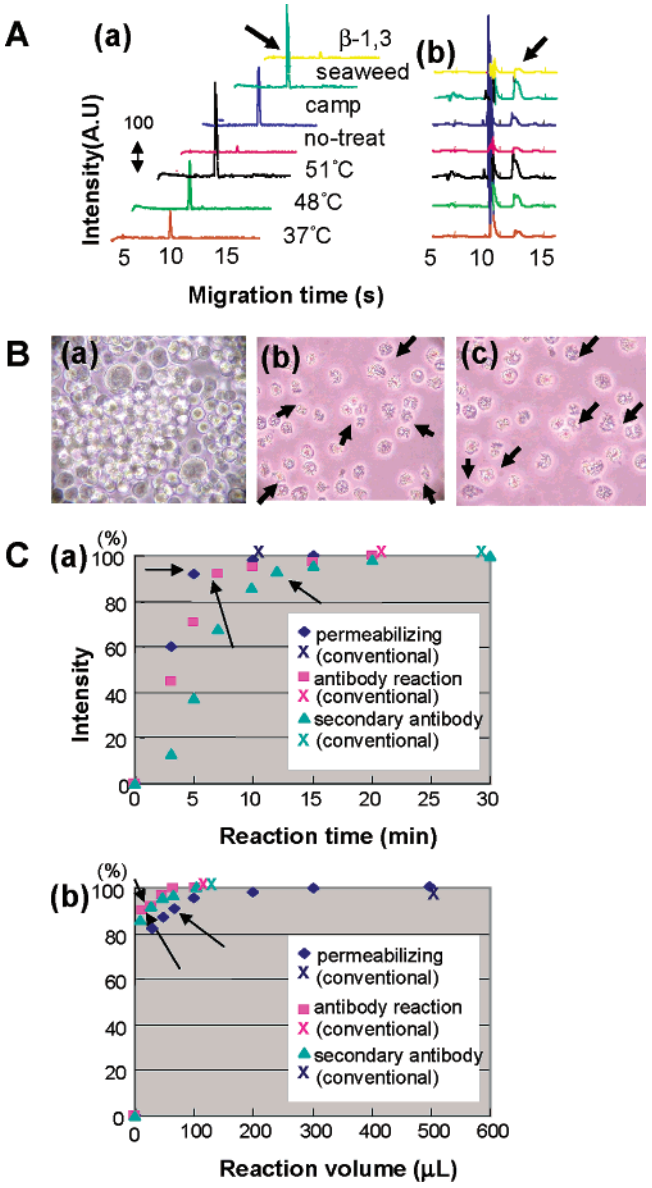

Figure 4. Analysis of specific proteins in an immunoassay induced by heat shock using the connected type of microdevice (Figure 1B) under optimized conditions determined from Figure 3C. (A) The electropherograms from the protein assay[20,21] of each specific protein using SV1210 (Hitach Electronics Engineering). (a) caspase 3 and (b) phosphatidyl serine. Arrows show each specific protein. (β-1,3: β-1,3 glucan, camp: camptothecin treated cells). (B) Microscopic observations of (a) heat-treated (51 °C), (b) camptothecin-treated, and (c) seaweed-treated Jurkat cells. Arrows show the cell fragmentation and degradation derived from apoptosis. (C) Optimization of the (a) reaction time and (b) reaction volume of the caspase 3 assay. Arrows show the minimum reaction times and reaction volume for each step. Conventional values (shown by the X characters) are recommended values of the manufacturer, which were adjusted to be 100% of intensity.

This seems inadequate for studying cancer cells because such cells prefer relatively high-density environments. Takano et al.[27] developed micropatterned substrates for probing intracellular communication pathways, but this system failed to include an immunoassay system.

Some commercially available assay kits, such as kits for apoptosis, require no laborious procedures. These kits are useful for protein analysis. However, such kits are restricted for use with particular assays. Berna et al.[28] established an automated sample preparation system, integrating collection, storage, and filtration using the Ansys Captiva 96-well filter plates. Tang et al.[29] and Morozov et al.[30] attained a HTS immunoassay system that incorporated a microtiter plate and multichannel electrochemical detection. This system is highly admired, but it failed to include the cell culture process.

Since our system requires no denaturing processes,[20] all of the pretreatment procedures for protein analysis can be readily integrated on it. To use other commercial electrophoresis separation chips, it is possible to choose the separated pretreatment chip (Figure 1A) and transfer the extracted proteins to the commercial electroseparation chip using a pipet only once. Our system is applicable to nearly all bioassays. Our system successfully integrates all procedures, such as cell culture, washing cells, stimulation, bioassay reaction, and electrophoretic separation. Our microdevice will be in high demand for HTS and may accelerate proteome research.

Conclusions

In summary, we have developed a self-contained on-chip cell culture and pretreatment system. 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 seaweed induced apoptosis of Jurkat 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 idiotypic for specific physiologic or pathologic states of cells.

Abbreviations: μ-CE, microchip electrophoresis; HTS, high-throughput screening; FBS, fetal bovine serum; MS, mass spectrometry.

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