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A microfluidic cell culture system for monitoring of sequential changes in endothelial cells after heat stress

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ABSTRACT

Endothelial damage induced by a highly elevated body temperature is crucial in some diseases including viral hemorrhagic fevers. Here, we report the heat-induced sequential changes of endothelial cells under shear stress, which were determined with a microfluidic culture system. Although live cell imaging showed only minor changes in the appearance of heat-treated cells, *Hsp70* mRNA expression analysis demonstrated that the endothelial cells in channels of the system responded well to heat treatment. F-actin staining also revealed clear changes in the bundles of actin filaments after heat treatment. Well-organized bundles of actin filaments in control cells disappeared in heat-treated cells cultured in the channel. Furthermore, the system enabled detection of sequential changes in plasminogen activator inhibitor-1 (PAI-1) secretion from endothelial cells. PAI-1 concentration in the effluent solution was significantly elevated for the first 15 min after initiation of heat treatment, and then decreased subsequently. This study provides fundamental information on heat-induced endothelial changes under shear stress and introduces a potent tool for analyzing endothelial secretions.

Introduction

A highly elevated body temperature can damage endothelial cells, leading to diffusion of serum proteins and blood cells and secretion changes of coagulation and aggregation inhibitors. Endothelial damage is thus implicated in causing symptoms such as increased vascular permeability, internal bleeding, and coagulation disorders. In particular, these symptoms are induced by some viral hemorrhagic fevers such as Ebola virus infection, Crimean-Congo hemorrhagic fever, and dengue hemorrhagic fever [1] (for review, [2,3]). The pathogenic mechanisms underlying the endothelial damage associated with these viral hemorrhagic fevers are very diverse and complex, including both direct and indirect dysfunction of endothelial cells [2]. The direct effects of elevated temperature and viral infection on endothelial cells are particularly important, and have been reviewed recently [4,5]. Other studies using animal models have suggested that endothelial cells are early targets of heat stress injury [6,7]. Although the importance of heat injuries on endothelial functions in these viral hemorrhagic fevers or other diseases that are accompanied by high fever has been long recognized, only a few *in vivo* and *in vitro* studies have focused specifically on the effects of heat stress on endothelial cells [8–10]. In addition, all previous *in vitro* reports were carried out using stationary culture methods with standard culture flasks, wells, or transwell cell culture inserts. However, since vascular endothelial cells *in vivo* are constantly subjected to shear stress associated with blood flow [11], it is likely that their responses in conventional stationary cultures *in vitro* do not properly reflect their responses *in vivo*.

Therefore, in order to examine the effect of heat stress on endothelial cells cultured under shear stress, we constructed a microfluidic culture system for endothelial cells, and monitored their morphological and secretion changes during heat stress. This system enabled detection of the heat-induced sequential changes in cytoskeletal actin filaments and secretion of plasminogen activator inhibitor 1 (PAI-1).

Materials and Methods

Cell Culture in Flasks

We used two endothelial cell lines: monkey RF/6A 135 cells (Cell Bank, RIKEN BioResource Center; Tsukuba, Japan) and human umbilical vein endothelial cells (HUVECs; Life Technologies). We used RF/6A 135 cells, which originate from monkey chorioretinal vessel endothelial cells, because overexpression of HSP70 is known to be easily induced in endothelial cells of chorioretinal vessels by increased temperature [12]. In addition, HUVECs were used to ensure the evaluation of monkey PAI-1 cells with the 'Human PAI-1 ELISA kit' employed in the present

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study. These cells were cultured in 75-cm^2 tissue culture flasks (Techno Plastic Products AG; Switzerland) at 37.5 °C in a humidified 5% CO_2 atmosphere in RPMI 1640 medium (MP Biomedicals, Japan) supplemented with 10% fetal bovine serum (FBS; Biological Industries; Kibbutz Beit Haemek, Israel), 50 IU/ml penicillin (Meiji Seika Pharma Co., Ltd.; Japan), and 25 μg/ml streptomycin (Meiji Seika Pharma Co., Ltd.; Japan) for RF/6A 135 cells, and in MCDB 107 endothelial basal medium (COSMO BIO Co., Ltd.) supplemented with 10% FBS, 50 μg/ml endothelial cell growth supplement (Takara Bio, Japan), 100 μg/ml heparin (heparin sodium, Wako Pure Chemical Industries, Ltd.; Japan), 50 IU/ml penicillin, and 25 μg/ml streptomycin for HUVECs.

To analyze the expression of *Hsp70* mRNA and PAI-1 secretion from cells cultured in flasks, RF/6A 135 cells were seeded into 12 culture flasks, six for controls (three for *Hsp70* mRNA analysis and three for PAI-1 analysis) and the other six for the heat-treated groups. After the cells were cultured at 37.5 °C overnight, they were incubated for 1 h at 42.5 °C (heat-treated group) or at 37.5 °C (control group). Thereafter, mRNA or the media were collected and analyzed as described below.

Cell Culture in the Channels of the Microfluidic Culture System

In this study, we used a microchip (Fig. 1A, Sumitomo Bakelite Co., Ltd.) with a parallel flow channel made of polystyrene (PS), as preliminary studies determined that this was the optimal chip material for culture of RF/6A 135 endothelial cells and HUVECs. A channel for fluidic cell culture was formed in the interior of the base PS plate. The base and cover PS plates, both of which were 1-mm thick, were thermally laminated. Each microchip had 2 channels, which were 6-cm long, 300-μm wide, and 100-μm deep (Fig. 1B). Before the cells were introduced into the channel, the microchip was sterilized with 70% ethyl alcohol and rinsed with sterilized distilled water. Then, each channel was pre-coated with collagen type IV solution (Nitta Gelatin Inc.; Japan) as follows. First, the channel was washed with 0.1 M NaOH, 70% ethanol, and sterilized distilled water in succession. Next, the inner surface of each channel was coated by filling it with collagen type IV solution for 2 h. The channels were then washed with sterilized distilled water five times and the chip platform was connected with sterilized tubes, valves, and syringes (Fig. 1C).

After filling the channels with culture medium, the inlet was closed. The microchip was then placed on the inserted heating plate (ITO temperature controller) set on the microscope stage (Nikon; Tokyo, Japan) (Fig. 1D). The temperature of the heating plate was set to 37.5 °C. Subconfluent endothelial cells were dissociated with papain (Worthington Biochemical Corporation; Lakewood, NJ, USA) and dispersed in the medium to prepare the cell suspension at a concentration of 2×10^7 cells/ml. We then introduced 35 μl of the cell suspension into the channel via the inlet. When the cells passed through the channel, the same cell suspension was also inserted into another channel. After seeding the cells, the inlet was closed and the microchip was immobilized for 2 h at 37.5 °C to allow the cells to attach to the bottom of the channel. After 2 h, the valve was turned to the medium port and perfusion was started at 0.2 μl/min at 37.5 °C. Then, after 1 h perfusion, the perfusion flow rate was gradually increased to 2 μl/min.

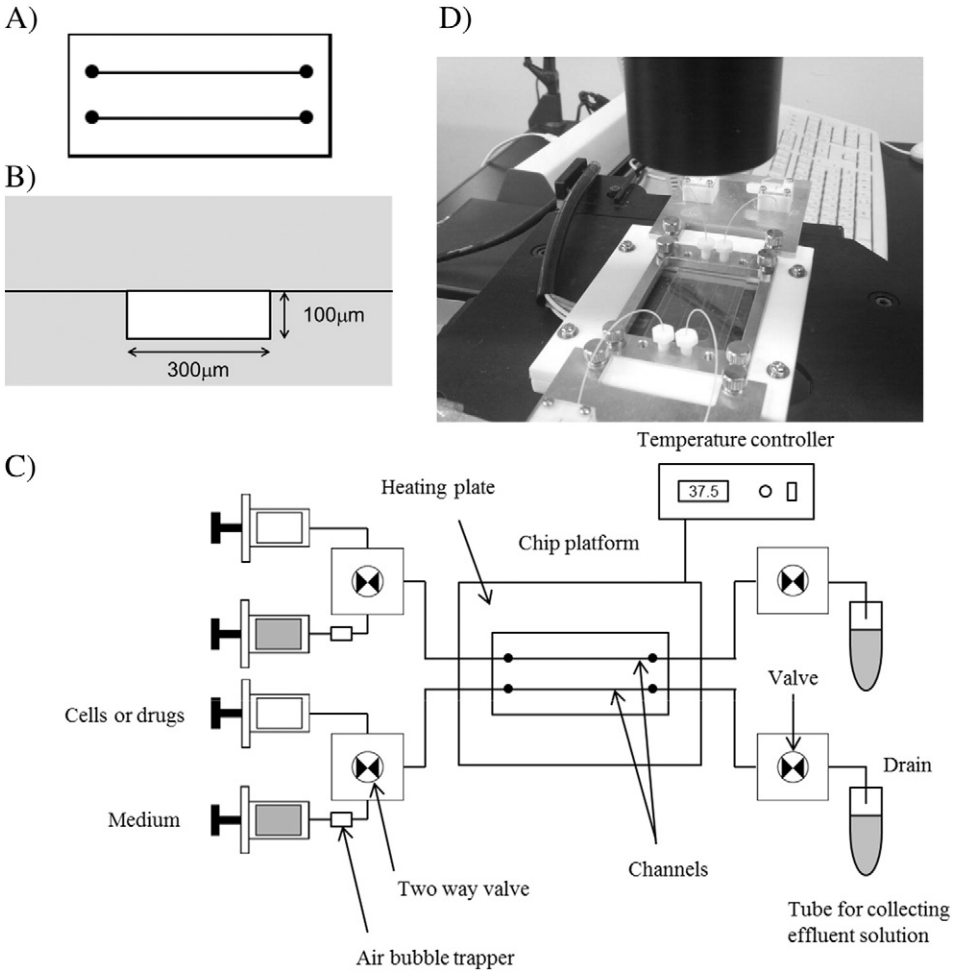

Fig. 1. A. Schematic illustration of the chip platform with two channels. B. Schematic diagram showing the partial cross-section of the channel inside the chip platform. C. Schematic illustration of the overall experimental setup of the microfluidic culture system. D. Image of the chip platform placed on the inserted heating plate set on the microscope stage.