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LOCALIZED EXPRESSION OF HEAT SHOCK PROTEIN IN CELL POPULATION BY MICRO HEATER DEVICE

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This paper reports on the localized expression of heat shock proteins (HSPs) in a cell population on a micron-scale. Thermal stimulation was realized by micro heaters on cells in microfluidic channels to investigate cell-cell interactions with precise temperature control of microfluidic channels utilizing temperature sensors.

Cell-cell interactions are considered as key factors in tissue and organ formation in vivo. Recently, some researchers locally stimulated cells using microfluidic devices and studied cell-cell interactions [1]. One of the most significant challenges in generating local stimulation is the precise confinement of the stimulants in microfluidic channels due to diffusion and flows. Our group has shown thermal stimulation on single cell level using micro heaters and observed heat shock responses (HSRs) [2]. Here, we present a micro heater device integrated with a microfluidic channel to control a temperature distribution locally on the cell culture medium. Well-controlled temperature distribution was achieved in a micro channel regulating the volume of culture medium and reducing natural convective heat transfer effects.

We fabricated the Pt micro heaters and temperature sensors using standard micro-fabrication process on glass substrates. Sensors were designed to measure the temperature from a change in electrical resistance due to high temperature coefficient of resistance (TCR) of Pt. Polydimethylsiloxane (PDMS) microfluidic channels were molded by SU-8 patterns. Finally, the micro heater chip was aligned and bonded to the PDMS micro channels. Figure 1 shows a heater and sensors aligned vertically to the micro channel. The sensors were designed in zigzag to have a higher resistance and thermal sensitivity as shown in figure 2. We designed the heater and sensors based on the finite element method (FEM) considering the surrounding substrate and medium. Then we generated the calibration curves shown in figure 2 and based on the curves, measured temperatures on the heater and sensors at different electric currents. For the cell culture process, the micro channel was coated by collagen after sterilization by ethanol and UV. NIH-3T3 fibroblasts that are transfected to express green fluorescent proteins (GFPs) with HSRs simultaneously [3] were confluently cultured in the channel. Finally, 15 mA was applied to the heater for one hour, and NIH-3T3 cells were locally stimulated by Joule heating.

Thermal sensitivities of the micro heater and sensor were calculated on fitting curves shown in figure 2 to be 0.053 and 0.087 Ω/K, respectively. As shown in figure 3, when 15 mA was applied to the micro heater, the temperature on a micro heater and a sensor reached 44°C and 41°C, respectively. Those experimental results agreed with simulation results shown in figure 4. As shown in figure 5, GFPs were observed symmetrically on the micro heater. The width of GFPs was 240 μm. This area was estimated to reach over 43°C from the simulation data.

A localized expression of HSPs was observed in a cell population by a micro heater device integrated with a micro channel. This device can locally stimulate cells and allow for the observation of cell-cell interactions through specific stresses on single cell level.

Word Count: 498

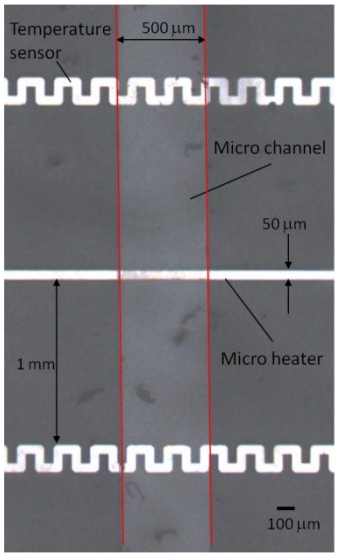


Figure 1. Optical image of Pt heater and temperature sensors aligned with the micro channel

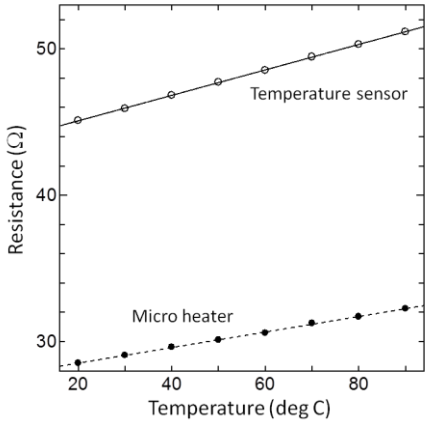


Figure 2. Calibration of Pt resistance measured at different temperatures

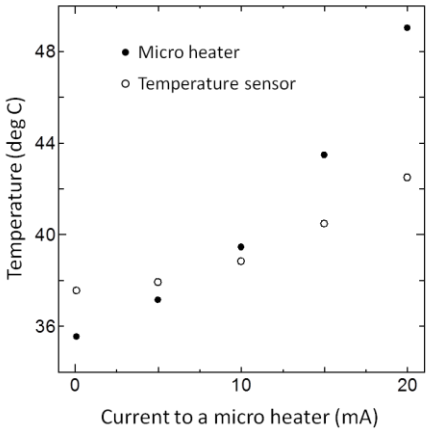


Figure 3. Temperatures on a micro heater and a temperature sensor measured by the calibration curves

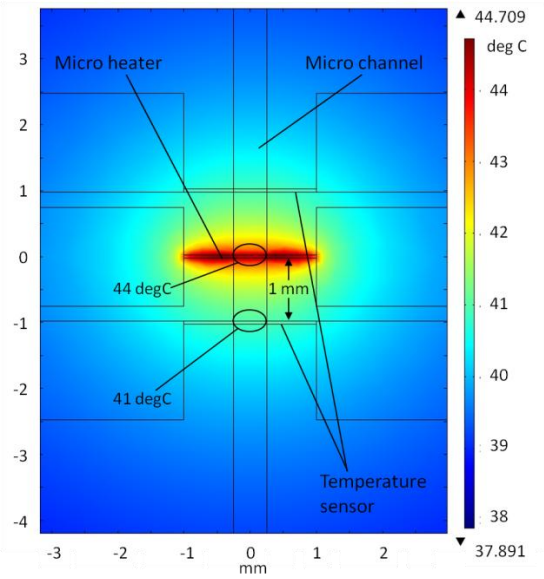
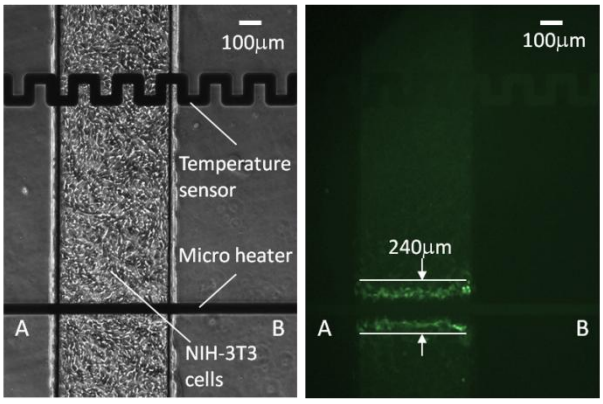


Figure 4. FEM simulation of the micro heater showing the temperature distribution on the glass substrate coverd by the PDMS micro channel



(a) *(b)*

Figure 5. (a) Optical image of NIH-3T3 cells eight hours later after heating taken by an inverted microscope (b) fluorescent image of GFPs expressed by NIH-3T3 (line AB shows the same micro heater)

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