PROLIFERATION OF COS-7 CELLS TRAPPED BY STANDING-WAVE ULTRASOUND IN A MICROFLUIDIC CHIP

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Abstract
We study cell viability after ultrasonic-standing-wave trapping of low cell numbers in a microfluidic chip by recultivation of the trapped cells. The cell proliferation rate is estimated by counting of initial and final cell numbers and shows normal cell growth. The results demonstrate the potential of ultrasonic standing waves as a tool for gentle and long-term manipulation of low cell numbers in microfluidic systems.

Keywords: Ultrasonic manipulation, cell handling, cell viability, image analysis

1. Introduction and theory

Ultrasonic-standing-wave (USW) technology has potential to become a standard method for gentle and contactless cell handling in microfluidic chips. Cells are trapped in the ultrasonic pressure nodal planes by the long-range acoustic force, which may be designed to perform elementary manipulation functions [1]. However, in order to perform biologically accurate experiments on the trapped cells it is necessary that the ultrasonic cell manipulation is gentle.

2. Experimental arrangement

We investigate viability and proliferation of adherent COS-7 cells exposed to long-term USWs in a temperature-regulated microfluidic chip by a combination of several indicators of the cell state, i.e., fluorescence assays and automatic image analysis. Fig. 1a shows the USW resonant cavity where channel dimensions and ultrasound frequency have been carefully matched. The cells form two-dimensional aggregates of low cell numbers ($<10^3$) inside the chip as shown in Fig. 1b. These aggregates are trapped against a continuous flow of either cell culture medium or assay buffer and may be imaged by fluorescence microscopy. High-NA label-free cell imaging can be performed in an alternative arrangement using obliquely coupled USW enabling transmission light microscopy. Fig. 2 demonstrates USW-induced line-aggregation for distinction and numbering of cells in high-throughput applications.

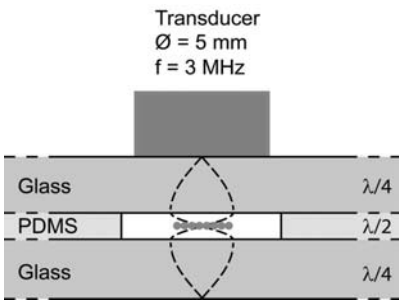

Figure 1a. Schematic of the chip.

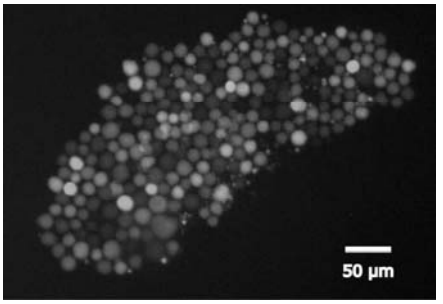

Figure 1b. Trapped two-dimensional fluorescently labeled cell aggregate.

Figure 2. COS-7 cells trapped in line aggregate in the new channel layout using obliquely coupled USW enabling the usage of transmitted light microcopy (20× objective).

To determine the cell state during USW exposure the cells were labeled with the direct viability indicator Calcein AM. For investigation of possible delayed cell damage we studied the proliferation rate of USW-exposed cell compared to non-treated control cells. Cell proliferation was measured by cell counting using image analysis tools after 2-3 days of cell cultivation (cf. Fig. 3) [2].

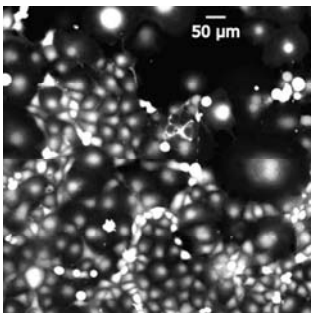

Figure 3. Recultivated COS-7 cells labeled with the viability indicator Calcein AM three days after USW exposure (partly overexposed).

3. Results and discussion

The cell growth after 2-3 days of recultivation, presented in Fig. 4, was estimated by comparing the initial and final number of cells. No deviations in the doubling time from expected values for adherent cells (24-48 h) were observed for COS-7 cells trapped at acoustic pressure amplitudes up to 0.85 MPa, and for trapping times up to 75 minutes. In contrast to the control cells, the USW-exposed cells also maintained the growth rate even at low cell numbers and cell concentrations. Thus, the results demonstrate the potential of ultrasonic standing waves as a tool for gentle and long-term manipulation of low cell numbers in microfluidic systems.

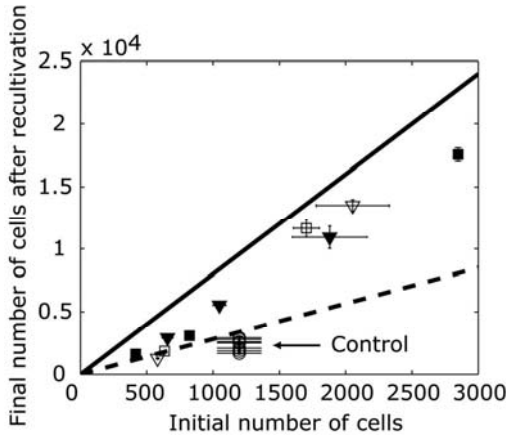

Figure 4. Cell growth was estimated by comparing initial and final cell numbers after 2-3 days of recultivation. Two experiments with USW exposure time of approx. 30 min [■,▼] and two with exposure times approx. 60 minutes were performed [□,▽]. Normal cell growth assuming doubling times of 24 [-] and 48 [--] hours are indicated. For comparison, untreated cells [○] were also cultivated.

Acknowledgements

This work was supported by the European Community-funded *CellPROM* project under the 6th Framework Program, contract No. NMP4-CT-2004-500039.

References

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