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Changes in stiffness of yeast cells during cell cycle by passability through micromachined channel arrays

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ABSTRACT

Possibility of cell sorting by cellular deformability was examined using yeast cells (*Saccharomyces cerevisiae*) and previously described microchannel arrays (width 6 μm , length 20 μm or width 8 μm , length 40 μm , depth 4.5 μm , and number 2600 or 4704 in parallel). Cells harvested at every an hour during incubation (YPD broth, 32°C, after preincubation for 24 hours) were washed and suspended in sorbitol (0.6 M) solution (pH 7.5) at a concentration of O.D. 0.3 (560 nm). An aliquot of each suspension was caused to flow through the microchannel arrays by applying 20 cmH_2O suction. Cells at two hours of incubation could not enter into the microchannels, while cells at 4-5 hours of incubation could enter into the microchannels despite their larger size due to budding than the preceding ones and some few cells were observed to pass through 8 μm width microchannels. The number of cells that could enter into the microchannels decreased at 7-8 hours and reincreased at 9-10 hours, but the synchronism in this second cycle appeared to decrease. Protoplasts prepared by treatment with zymolyase from cells at 4-5 hours of incubation showed no appreciable resistance to the microchannel passage.

Keywords: microchannel array, cellular deformability, cell cycle, cell sorting, *Saccharomyces cerevisiae*

1. INTRODUCTION

Cells in a given species or population have still differences in cellular state such as phase in cell cycle and activity or function. Separation of cells according to these differences is increasingly required in cell science and, particularly, in biotechnology which aims to effectively use cellular functions for artificial objectives.

Efforts have been made for decades to develop such a cell sorting method using sheath flow technique which produces center-line flow of cells and detection of laser-light scattering or laser-light excited fluorescence emission from the flowing cells^{1, 2}. The use of fluorescence-labeled monoclonal antibodies in this method has made it possible to separate cells due to differences in the amount of particular antigens expressed in them. However, there is a neck in this method caused by difficulties in creating monoclonal antibodies and also in keeping their binding force constant.

Mechanical properties of cells such as cellular deformability or stiffness, adhesiveness, and motility have been shown to be closely or inseparably associated with their physiological state and function. For example, erythrocyte deformability plays a pivotal role in their passage through capillaries³, and leukocytes change from a nonadhesive and nonmotile cell to an adhesive and motile cell when activated by chemotactic stimulation⁴. It is, therefore, of essential importance to characterize cells by these function-related mechanical properties, and it further appears that differences in such cell mechanical properties may be usable for the required separation of cells. However, quantitative mechanical measurements or measures are most difficult to give for cells by presently available techniques because of micrometer dimensions and undefinable shape of the object.

Micromachining⁵ would have most useful applications in giving tools having required dimensional accuracy for measurements and handling of cells. In previous studies, we have developed microchannel arrays for use in studies of blood rheology as a model of arrays of physiological capillaries⁶⁻⁹. In a preceding paper¹⁰, marked differences in flow characteristics through the microchannel arrays were described for resting and activated leukocytes. The aim of the present report is to describe changes during cell cycle in passability through the microchannel arrays for yeast (*Saccharomyces cerevisiae*), which is biotechnologically important in food science and industry, in the context of cell characterization and separation. Drastic changes in the microchannel passability are also shown for cells treated with ethanol and zymolyase.

2. MATERIALS AND METHODS

a) Microchannel arrays

The microchannel arrays used in the present study are those previously described in detail elsewhere^{7, 10}. Their principal structure is shown in Fig. 1. In this structure, microgrooves formed in the (100) surface of a single-crystal silicon substrate are converted to leak-proof microchannels by tightly covering them with an optically-flat glass plate. This structure made it possible to microscopically observe flow behavior of cells through the entire microchannel. The contact between the silicon substrate surface and the glass plate surface could be made water-tight by mechanical pressing alone because of optical flatness of the both surfaces.

Dimensions of microchannels (microgrooves) used are 6, 7, and 8 μm in width, 20, 30, and 40 μm in length, respectively, and 4.5 μm in depth, with 30 μm -wide terrace approaches in both entrance and exit sides. In our original design^{6, 7}, microgrooves of number 2600 were arranged in parallel as shown in Fig. 2a in a chip area dimensioned 15 x 15 mm. In a recent modification¹⁰, 4704 microgrooves were arranged more effectively as shown in Fig. 2b in a chip area dimensioned 12 x 12 mm.

Microchannels of width 6 μm and length 20 μm and width 8 μm and length 40 μm were used in the present study.

b) Macrofluidics

Because of the large numbers of microchannels arranged in parallel, the total fluid flow rate through all the microchannels ranges in $\mu\text{l/sec}$ and, hence, the present microchannel arrays can be used in macrofluidics without any interface. The macrofluidics actually used in the present study, shown in Fig. 3, has also been given in a previous report¹⁰. An aliquot of a suspension of cells is introduced into the sample cylinder and caused to

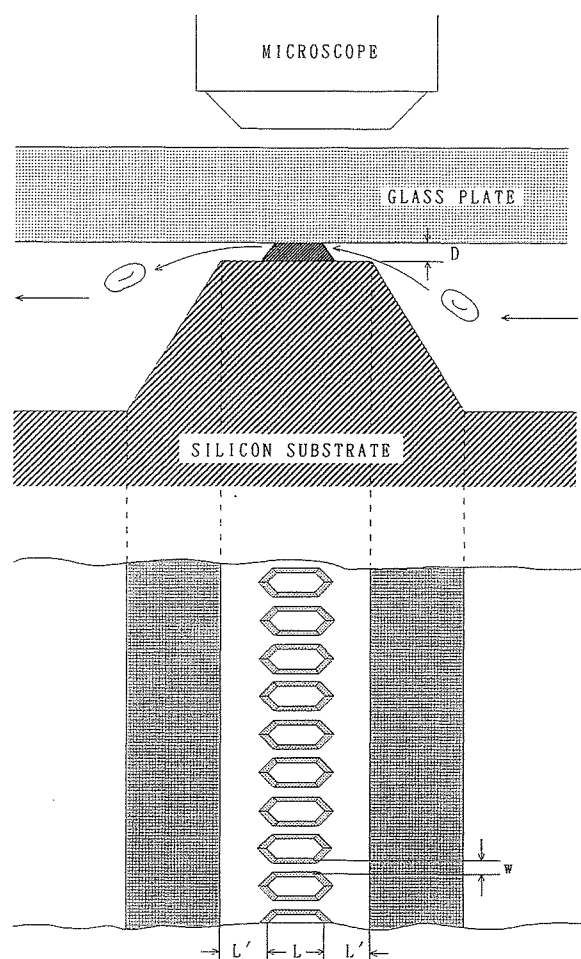
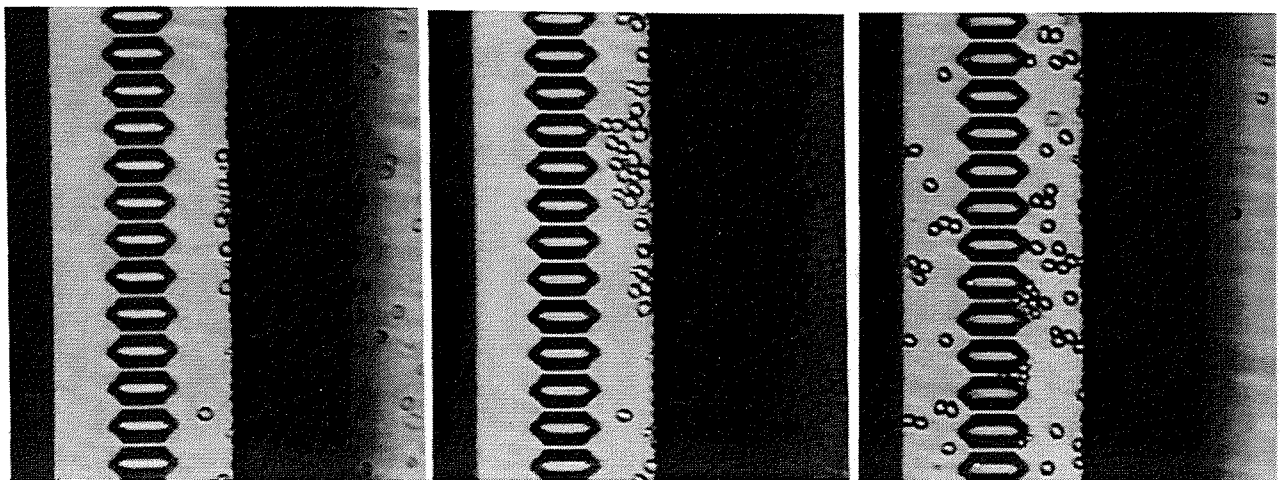


Fig. 1. Outline structure of microchannels formed between the surfaces of a single-crystal silicon substrate and of a glass plate.



a. 20 cmH₂O suction pressure. b. 200 cmH₂O suction pressure. c. cells exposed to 60 % ethanol (20 cmH₂O suction pressure)

Fig. 4. Passage of non-vegetative yeast cells through an array of 6 μm-wide, 20 μm-long microchannels.

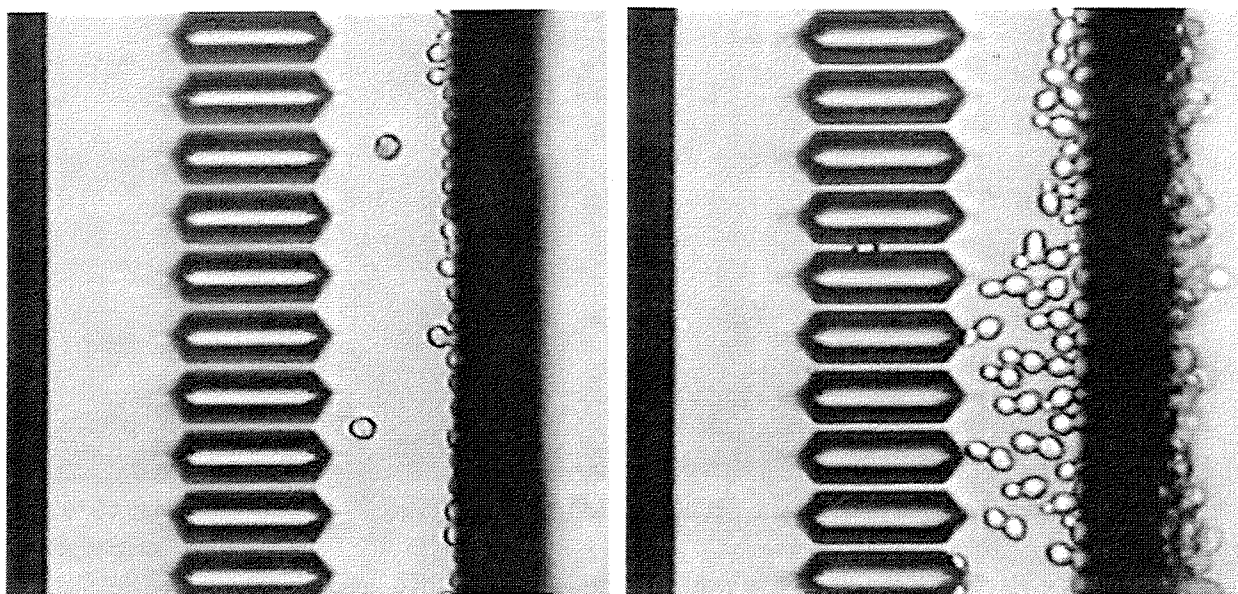
harvested at every an hour during the incubation were washed and suspended in 0.6 M sorbitol, 50 mM Tris-HCl (pH 7.5) solution at a concentration of O.D. 0.3 (560 nm). Protoplasts were prepared from vegetative cells at the exponential growth phase, i.e. cells incubated for 4-5 hours, as follows. Cells harvested were washed twice with 50 mM Tris-HCl (pH 7.5) solution (3000 rpm, 5 min) and suspended in 0.6 M sorbitol, 50 mM Tris-HCl (pH 7.5) solution containing 50 U/ml zymolyase (Biochemical Industry, Japan) and 0.2% 2-mercaptethanol. The suspension was gently agitated for 30 min at 32°C, and then the cells were washed twice with 0.6 M sorbitol, 50 mM Tris-HCl (pH 7.5) solution (1500 rpm, 10 min) and suspended in the same buffer at a concentration of O.D. 0.3 (560 nm). Cells obtained with varying reaction time from 10 to 70 min were also examined with the microchannel passability.

3. RESULTS

Non-vegetative cells could not enter into the entrance terrace approach under the suction pressure of 20 cmH₂O (Fig. 4a). They could not pass through the microchannels even under a 10-fold increased suction of 200 cmH₂O (Fig. 4b). Those cells became passable through 6 μm-wide microchannels under 20 cmH₂O when exposed to 60% ethanol, i.e., when suspended in 60% ethanol solution (Fig. 4c).

Vegetative cells showed changes in stiffness or deformability during the incubation. Cells harvested at 2 hours of incubation could not enter into the entrance terrace approach (Fig. 5a) as similarly as non-vegetative cells. Meanwhile, cells harvested at 4-5 hours of incubation could enter into the terrace portion despite their larger size due to budding than the preceding cells, and some few cells were observed to pass through 8 μm-wide microchannels under 20 cmH₂O (Fig. 5b). The number of cells that could enter into the terrace portion decreased at 7-8 hours and reincreased at 9-10 hours, but the synchronism in this second cycle appeared to decrease.

Fig. 6 shows two examples of changes during the incubation in the cell suspension passage time through the array of 6 μm-wide microchannels (4704 in parallel) under 20 cmH₂O suction. As shown, the cell suspension passage time increased at 3 hours and then decreased considerably till 6 hours and, after that, showed a reincrease and redecree, i.e., a cyclic change.



a. cells harvested at 2 hours of incubation

b. cells harvested at 5 hours of incubation

Fig. 5. Passage of vegetative cells through an array of 8 μm -wide, 40 μm -long microchannels under 20 cmH_2O .

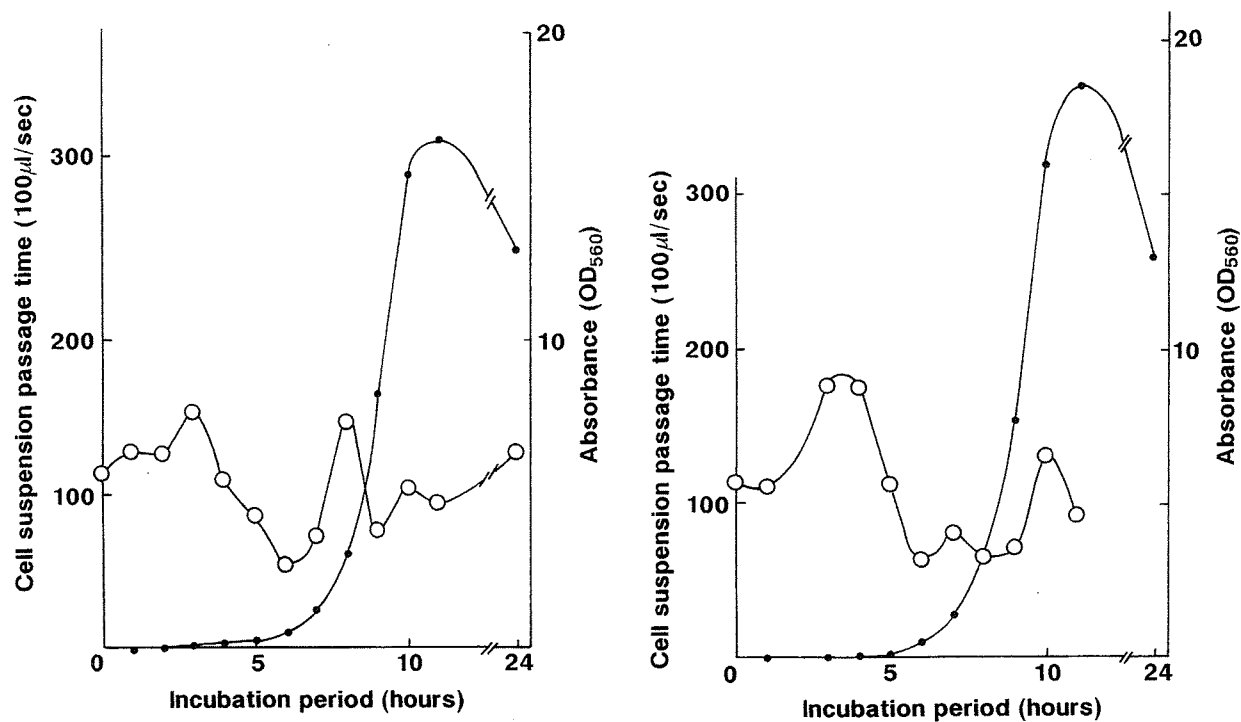


Fig. 6. Two examples of changes during incubation in the cell suspension passage time through an array (4704 in parallel) of 6 μm -wide, 20 μm -long microchannels under 20 cmH_2O suction.

Fig. 7 shows a change due to the zymolyase treatment in the cell suspension passage time through the same microchannel array as used in Fig. 6. The cell suspension passage time decreased steeply till 10 min of reaction

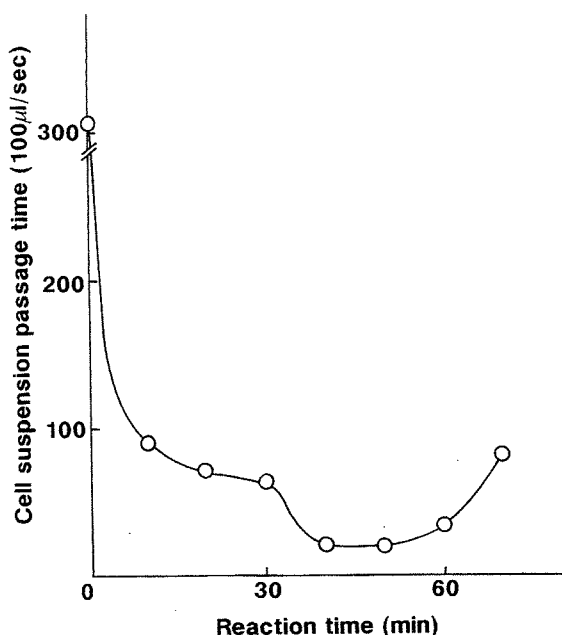


Fig. 7. Changes due to treatment with zymolyase in the cell suspension passage time through an array (4704 in parallel) of 6 μm -wide, 20 μm -long microchannels under 20 cmH_2O suction.

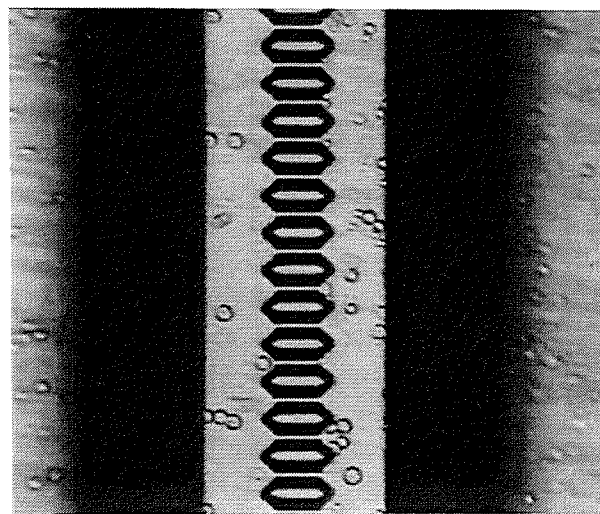


Fig. 8. Flow of protoplasts through an array of 6 μm -wide, 20 μm -long microchannels under 20 cmH_2O suction.

time, then relatively gradually till 30 min, and, showed a further decrease, that might be of different mode from the preceding one, from 30 min till 60 min. A reincrease was observed in the cell suspension passage time after 60 min. Cells obtained after 30 min treatment with zymolyase showed no appreciable resistance to the microchannel passage (Fig. 8).

4. DISCUSSION

We have so far studied flow of blood cells through the present microchannels. An extremely quick passage, i.e. an extremely high deformability, can easily be demonstrated for erythrocytes. For example, an estimate of mean passage time of 0.4 msec has been given for human normal cells passing through the 6 μm -wide, 20 μm -long microchannels under the 20 cmH_2O pressure difference. Leukocytes are much less deformable than erythrocytes, but they still can squeeze well through the microchannels; estimates about 300 times larger, on average, than those of erythrocytes have been given for their passage time through the microchannels of the same size under the same pressure difference as those given above. As aforementioned, activated leukocytes show a further increased resistance to the microchannel passage.

Despite a smaller size than erythrocytes and leukocytes, no passage was observed in most cases for yeast cells using the present microchannels and even a 10-fold increased suction pressure. Such a stiffness or rigidity is obviously attributable to that these cells possess a rigid cell wall. As demonstrated in Fig. 8, protoplasts, i.e. cells deprived of the cell wall, showed no appreciable resistance to the microchannel passage.

The most interesting finding we have obtained in the present study is that the stiffness of the cell wall may change during the cell cycle. As clearly shown in Fig. 5, it appears that cells that are budding are softer than cells in the preceding phase of the cell cycle. This appears to be natural as budding would be otherwise impossible to make. It further appears to be possible from the present observation to effectively separate

budding cells from cells in the other phases of the cell cycle using microchannels of depth 4.7-4.8 μm and width 9-10 μm at the middle point of the depth.

The following comment should be given to the cell suspension passage time used in Fig. 6. This was actually the passage time of suspending fluid through intercellular spaces in piled cells in the upward stream of the microchannel array. If softening of the cells results in their closer packing, this should increase the fluid passage time. Therefore, the increased passage time obtained at 3 hours of incubation does not mean hardening of the cells but probably means an opposite softening of the cells. The marked decrease in the passage time obtained from 3 till 6 hours would also include increased intercellular spaces in the cell pile due to buds formed.

Such an effect of the cell shape and softness should also modify the change in the cell suspension passage time with the cell treatment with zymolyase shown in Fig. 7.

The two phases of the decrease notable in the cell suspension passage time shown in Fig. 7 might be explained by a possible difference in the microchannel passage between protoplasts and spheroplasts, i.e. cells partially retaining cell wall.

Non-vegetative yeast cells gained passability through the present microchannels when they were exposed to 60 % ethanol (Fig. 4c). This change might be attributable to their probable shrinkage by ethanol. However, such a volume change was unclear from the microscopic observation and, furthermore, intact cells have already a distribution in cell volume. It is therefore difficult to explain the passability that all the cells gained by the probable volume change. Some components of the cell wall might be extracted by ethanol, and this might result in softening of the cell wall.

In conclusion, we have shown changes in stiffness of yeast cells during cell cycle by examining their passability through micromachined channels. It further appeared to be possible to sort cells by the microchannel passability.

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