SINGLE CELL TRAPPING AND ANALYSIS OF PROKARYOTIC PRODUCTION STRAINS IN SUB-µm FLUIDIC STRUCTURES

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ABSTRACT

A disposable microfluidic chip system for single cell analysis is presented. The device enables hydrodynamic trapping of single bacteria cells of various industrial prokaryotic strains, e.g., *E. coli* and *C. glutamicum*, in partly sub-µm fluidic structures. Single cell traps are arranged in arrays aiming for quasi high-throughput cell observation with respect to cell growth, stress stimuli and productivity. High resolution live cell imaging microscopy is performed in a time lapse manner allowing the observation of multiple ROI. This work paves the way for future system biological and metabolic investigations such as single cell and cell-to-cell heterogeneity studies with the main goal of collecting statistical data for relevant parameters in biotechnology. As a proof of principle, the successful trapping and cultivation of single *E. coli* and *C. glutamicum* bacteria cells within a laminar flow, as well as fluorescence based specific amino-acid detection on single cell level is demonstrated in this contribution.

KEYWORDS: Single Cell Analysis, Hydrodynamic Trapping, Systems Biology, Bacteria, *E. coli*

INTRODUCTION

Bioprocess optimization of large-scale fermentation processes is an ongoing field of interest. Since the last decade there is a rising interest in how and if cellular heterogeneities influence large scale-fermentation processes. In a reactor it is estimated that cells are in many different states, due to genetic and non-genetic heterogeneity but although through environmental heterogeneities based on large-scale bioreactor design [1,2]. To investigate the behavior as well as state of single cells, a well-established method is flow cytometry in combination with fluorescence activated cell sorting (FACS). FACS is an ideal high throughput tool for screening and sorting of cellular populations [3]. However, FACS is a snapshot analysis and the investigation of time dependent processes is limited. Furthermore, environmental heterogeneities are not taken into consideration in FACS studies. In contrast, microfluidic cell analysis and especially single cell analysis offer new perspectives to investigate time-dependent processes of both, cellular and environmental heterogeneity.

Microfluidic single cell analysis has been demonstrated since a few years and is a known research field mostly for eukaryotic cells [4,5]. It is obvious, that relatively large eukaryotic cells, typically 10 to 100 times larger than prokaryotic cells, put less demand on chip fabrication and on cell manipulation when aiming for single cell observation. In addition, the relatively fast exponential growth behavior and in some cases biofilm formation operate against single cell analysis. Due to that, the field of single cell trapping and analysis of prokaryotic bacteria cells, typically with sizes in the order of few to several micrometer, is still in its infancy. Especially the analysis of growth and production behavior of industrially used bacteria was to our best knowledge not reported before.

Shown in literature, first hydrodynamic bacteria trapping structures were adapted from eukaryotic cell traps and are suitable to investigate small cell populations rather than single cells [6]. A similar system, having growth channels to study growth behavior of *E. coli*, was developed by Wang *et al.* [7]. Wang's system was used for system biological growth experiments of different *E. coli* strains. The drawback of the system is the difficulty to investigate industrial non-motile bacteria, since the filling relies most likely on motile bacteria strains.

PRINCIPLE

The system shown here is specially designed for parallel single cell experiments to determine cell growth, stress resistivity and productivity of industrial bacteria. Sub-µm traps were developed and fabricated which are a factor 10 smaller than existing traps allowing the long-term investigation of single bacteria cells, e.g., without nutrient deprivation. Furthermore, our system enables real-time fluorescence measurements by having all bacteria in one focal plane. In-house developed industrial productions strains mainly based on *E. coli* and *C. glutamicum* are used for first proof of principle experiments.

A two layer configuration is applied, having media supply channels with 8 µm height and cell trapping arrays with 800 nm channel height. This geometry enables cell growth within one focal plane, ideal for live cell imaging microscopy. Cells are loaded into the traps hydrodynamically with the fluid flow dragging cells into the traps. Once a trap is loaded, the laminar flow is diverged around the trap, ideally not trapping any additional cells. Excess bacteria as well as appearing daughter cells are supposed to be dragged away and removed by the continuously flowing media. Figure 1 shows the principal of the single cell trapping (Figure 1 A) and the flow profile of a single trap (Figure 1 B).

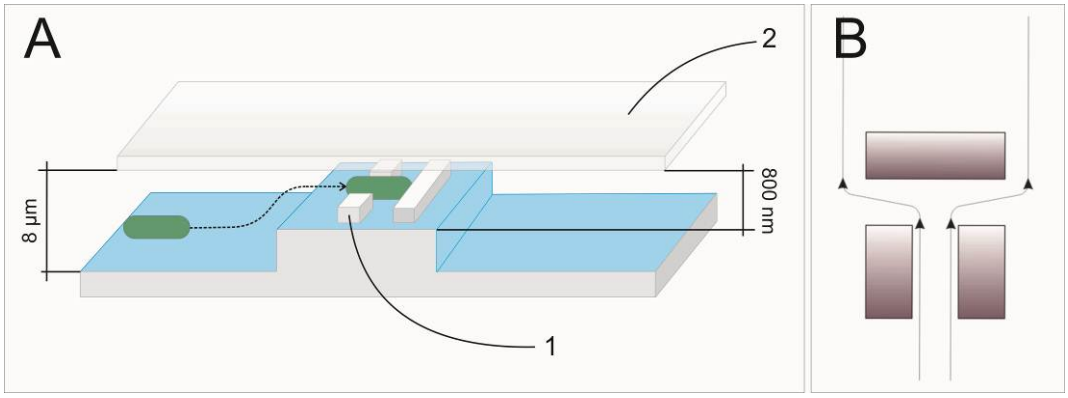

Figure 1: Illustration of the microfluidic device. (A) Chip and trapping structures are made of PDMS (1). The chip is sealed with a 170 µm thick glass plate suitable for high NA microscopy (2). The main channel is 8 µm in height, whereas the trapping area has a height of 800 nm enabling cell growth within one focal plane, ideal for time lapse microscopy (B) Laminar flow profile through a single cell bacteria trap.

EXPERIMENTAL

Using common soft-lithographic methods, a disposable poly(dimethylsiloxane) (PDMS) microfluidic chip was fabricated [8]. As shown in Figure 2, each chip of app. 20mm x 25mm in size contains several trapping arrays. To verify structure geometry and wall roughness SEM was performed on a trapping array and a single trap with the images shown in Figure 2 (middle, left). Various single cell trap designs were developed, differing in length and width. These are currently under further investigation to cope with different morphology of genetically modified production organisms.

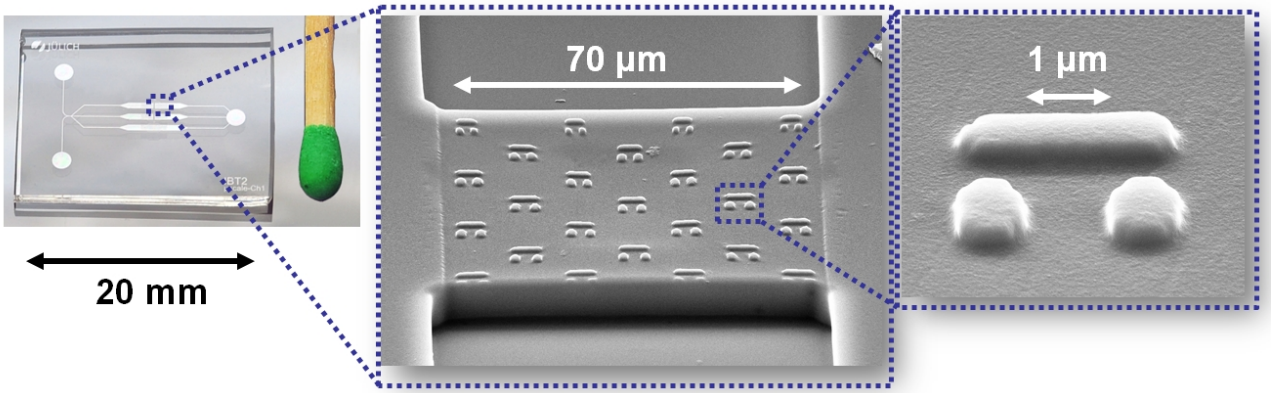

Figure 2: Photographs of the chip and trapping structure: (left) PDMS chip containing in- and outlets and 3 channels containing 5 trapping arrays each; (middle) SEM scan of a single trapping array containing several single cell traps; (right) SEM scan of a one single cell trap, 3 µm in width and length and app. 800 nm in height.

RESULTS AND DISCUSSION

Figure 3 (left) shows a selection of a time-lapse growth experiment of *E. coli BL21* cultured at 37°C in LB- media with a media flow rate of app. 50 nl/min at one cell array. The selected figures illustrate one cell cycle of a continuously growing *E. coli* cell. It can be seen that the growth of a single bacterium is linear (Figure 3 right). It is continuously growing during the entire growth cycle (cell growth and cell division). Figure 4 shows two cells with an EYFP based specific amino-acid sensor which was activated on chip through dipeptide injection after trapping and division. Unfortunately, here two cells were trapped in parallel, indicating further need for design optimization.

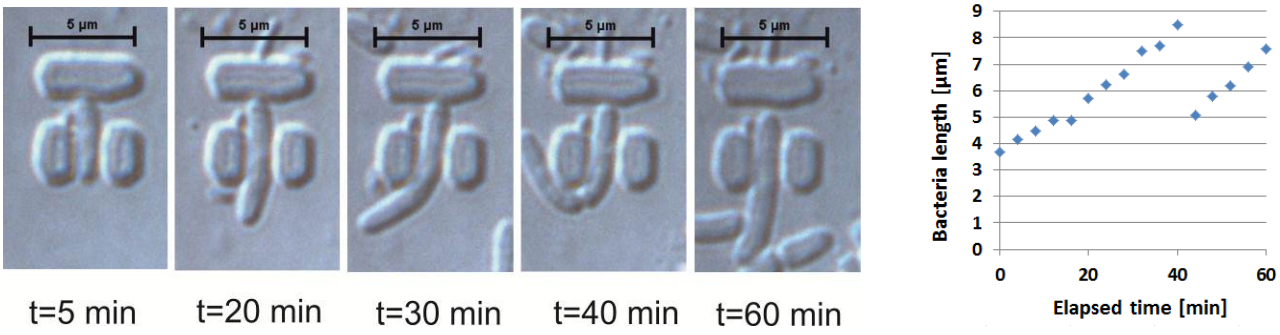

Figure 3: (left) Selection of a long-term growth experiment of bacteria, showing a single trapped *E. coli* cell (t=5 min), which is growing and dividing after t≈40 minutes. After cell division the old-pole cell continues growing (t=60 min).(right) Growth curve of the single cell growth illustrated on the left side.

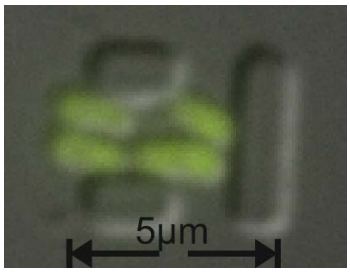

Figure 4: Trapped *C. glutamicum cells*, showing fluorescence triggered by induction.

CONCLUSION

The proof of principle of trapping bacteria on a single cell level and the real-time observation of cell division of several divisions for two different industrially used organisms was successfully performed. The developed system opens up new possibilities for future studies on single cell growth behavior of industrial bacteria and for detailed analysis of production on single cell level through fluorescence based metabolite sensors. Scale up and automation of this device to perform multiple single growth and production experiments, is currently in progress. Potentially the system can be applied to system biological questions on single cell level. Furthermore, the system will be used for large scale bioprocess development such as process optimization and scale-down studies.

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REFERENCES

[1] Huh, D. and J. Paulsson, *Non-genetic heterogeneity from stochastic partitioning at cell division.* Nature Genetics, 2011. **43**(2): p. 95-U32.
[2] Lara, A.R., et al., *Living with heterogeneities in bioreactors.* Molecular Biotechnology, 2006. **34**(3): p. 355-381.
[3] Davey, H.M. and D.B. Kell, *Flow cytometry and cell sorting of heterogeneous microbial populations: The importance of single-cell analyses.* Microbiological Reviews, 1996. **60**(4): p. 641-&.
[4] Danino, T., et al., *A synchronized quorum of genetic clocks.* Nature, 2010. **463**(7279): p. 326-330.
[5] Di Carlo, D.W., LizY.; Lee, LukeP., *Dynamic single cell culture array.* The Royal Society of Chemistry, 2006.
[6] Kim, M.-C., et al., *Programmed trapping of individual bacteria using micrometre-size sieves.* Lab on a Chip, 2011. **11**(6): p. 1089-1095
[7] Wang, P., et al., *Robust Growth of Escherichia coli.* Current Biology, 2010. **20**(12): p. 1099-1103
[8] Xia, Y.N. and G.M. Whitesides, *Soft lithography.* Annual Review of Materials Science, 1998. **28**: p. 153-184.

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