Capital Biochip Corporation
National Engineering Research Center
for Beijing Biochip Technology

A Novel Miniaturized Cell Lysis Device Using Spherically Focused Ultrasound

Gang Li[1,2] , Hong Xiao[1,2], Min Guo[3] and Jing Cheng[1,2,3]

[1]Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, P. R. China
[2]National Engineering Research Center for Beijing Biochip Technology, Beijing 100084, P. R. China
[3]Capital Biochip Corporation, Beijing 100084, P. R. China

Abstract

In this study, a prototype of miniaturized cell lysis device has been developed using a spherically concave transducer, which is capable of lysing bacteria in absence of added chemical denaturants, enzymes or microparticles and lysing yeast efficiently without any mechanical or enzymatic pretreatment. It is designed for miniature bio-analysis systems where cell lysing is needed to obtain intracellular materials for further analysis such as DNA identification. The lysis efficiency of the device was evaluated by viable cell counts and microscopy. Additionally, we compared its efficiency with that of traditional chemical cell lysis method using standard molecular biological techniques such as agarose gels and UV (ultra-violet) spectroscopy. Results from our experiments indicated that efficient bacteria and cell disruption could be achieved through a spherically focused transducer with a low voltage and high frequency.

Introduction

Increasing research efforts are directed toward the miniaturization of biological analysis systems and their integration. The total analysis system capable of sample in/answer out operation will require multiple functions including sample preparation, sample component discrimination, and detection/data analysis. To date, a large majority of the technical efforts relating to sample preparation have been invested in the development of the sample post-preparation (including amplification) component of the various analysis systems such as capillary electrophoresis or the chambers used for miniaturized polymerase chain reaction (PCR) systems. Few efforts have been directed toward the development of technologies for micro scale sample pre-preparation. Hence, the objective of our work is to develop a rapid, easy-to-integrated device capable of lysing cells with rigid wall such as bacteria and yeast in the absence of added chemical denaturants, enzymes, or particles, with liberated nucleic acids available for subsequent nucleic acid purification and detection. The resulting device was designed to focus megahertz ultrasonic energy on a microcentrifuge tube or a flow cell for cell lysis (as shown in Fig. 1)

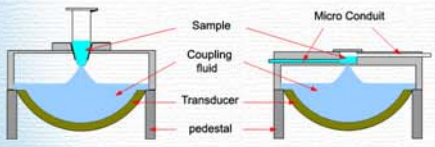

Fig. 1 Illustration of two spherically focused sonicators

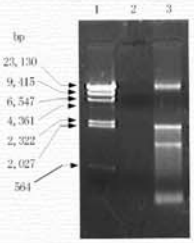

Fig. 2 Agarose gel electrophoresis of nucleic acids released from *E. coli* after applying the spherically focused ultrasound. Lane 1: λDNA Hind III digest marker, Lane 2: control with no treatment, Lane 3: the ultrasonication lysate with a time span of 30sec and acoustic intensity of 5.2W/cm²

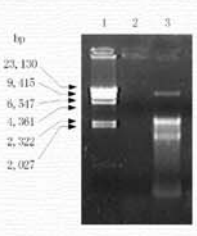

Fig. 3 Agarose gel electrophoresis of nucleic acids released from yeast cells after applying the spherically focused ultrasound. Lane 1: λDNA Hind III digest marker, Lane 2: control with no treatment, Lane 3: the ultrasonication lysate with a time span of 30sec and acoustic intensity of 5.2W/cm² (plus lysis buffer)

Table 1. Percent Loss of Viability for *E.coli* and *S. cerevisiae* after treatment of spherically focused ultrasound

organism	Loss in viability (%)[a,b]
E. coli	99.93
S. cerevisiae	99.13

[a]Cells were treated with the acoustic intensity of 5.2W/cm² for 30sec. [b]Values are the arithmetic mean from three trials and nine plate counts.

Table 2. A comparison of DNA availability of *E. coli* by ultrasonication and chemical method

method	Yield(μ g/ml)[b]
Sonication[a]	6.55
Chemical method	6.83

[a]Cells were treated with the acoustic intensity of 5.2W/cm² for 30sec. [b]Values are the arithmetic mean from three trials.

Result and Discussion

E. coli (strain HB101) and *S. cerevisiae* were selected to test the efficacy of the spherically focused sonicator. Results from the plate counts experiments (Table 1) indicated the bacteria and yeast disruption could be obtained by applying a spherically focused ultrasound. Moreover, the validity of sonicator was confirmed by the release of intracellular materials, which was determined by agarose gel electrophoresis method (as shown in Fig. 2 and Fig. 3). Additionally, we also quantitively assessed the availability of nucleic acid from *E. coli* after ultrasonic treatment by UV spectroscopy (Table 2). Results showed the yield of DNA with the ultrasonication method was comparable to that with the traditional chemical lysis method.

Conclusion

In this study, we have demonstrated that spherically focused ultrasound can be used to rapidly and efficiently disrupt *E. coli* in the absence of any physical or chemical pretreatment. Although ultrasonication alone is not enough to effectively disrupt yeast cells, it can significantly shorten the lysis time and simplify the lysis process of yeast cells when combined with lysis buffer or organic solvents.

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*If any questions, please feel free to contact me by gangli02@hotmail.com