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ORIGINAL PAPER

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Development of a laser-induced cell lysis system

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Abstract A novel cell lysis system was developed that is based on laser-induced disruption of bacterial and yeast cells. It will find application as a rapid, efficient and clean sample preparation step in bioanalytical detection systems. Using *E. coli* as our model analyte, we optimized cell lysis with respect to optimal laser wavelength, lowest energy input requirements, RNA release from the cells, and potential protein damage. The optimized system was finally applied to the lysis of four additional microorganisms. All experiments were carried out with about 2000 cells per sample or less. Initially, lysis was determined by the detection of cell survival after laser treatment using standard microbiological techniques, (i.e., cells were grown on nutrient agar plates). Then, actual release of mRNA from the cells was proven. Wavelengths investigated ranged from 500 nm to 1550 nm. An average power of 100 mW for the lasers was shown to be sufficient to obtain cell lysis at wavelengths above 1000 nm, with optimal wavelengths between 1250 nm and 1550 nm. Since water absorbs energy at those wavelengths, it is assumed that laser exposure results in an instantaneous increase of the cell temperature, which causes rupture of the cell membrane. Second, damage to protein solutions treated under optimized laser-lysis conditions was also studied. Using a pure solution of horseradish peroxidase as a model protein, no loss in enzyme activity was observed. Thus, it was concluded that damage to intracellular proteins is unlikely. Third, RNA release was tested using an *E. coli* specific RNA biosensor. Release of RNA was not detected from untreated cells, but laser-treated *E. coli* cells displayed significant RNA release due to laser-induced cell lysis. Finally, lysis of *M. luteus*, *B. subtilis*, *B. cereus*, and *S. cerevisiae* were investigated under optimized conditions. In all cases, laser-induced lysis of the cells was confirmed by determination of cell survival. Hence, laser-induced cell lysis is an efficient procedure that can be used for sample preparation, without damage to macromolecules, in bioanalytical detection systems for microorganisms. Miniaturized lasers and miniaturized cell-lysis chambers will create a simple, field-usable cell lysis system and allow the application of laser-induced cell lysis in micro Total Analysis Systems.

Keywords Laser · Cell lysis · Biosensor · Pathogenic organism · E. coli

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Introduction

Bacterial pathogenesis is one of the most challenging worldwide problems making it necessary to analyze medical, food, and environmental samples for the presence of pathogenic microorganisms. Worldwide, infectious diseases account for 40% of the estimated total 50 million annual deaths. A critical step in detection and identification of pathogenic organisms is very often cell lysis. In order to determine intracellular proteins, DNA, or RNA molecules, a cell membrane has to be disrupted via a rapid, non-damaging, and simple procedure, which ideally does not add adulterating chemicals into the cell lysate or further dilutes the sample. Each sample preparation step in a bioanalytical detection system needs to be designed carefully, especially if single cell detection is targeted. It can be expected that technology for the manipulation and analysis of single cells will play an important role in such areas as biomedical research [1, 2], drug discovery, diagnosis of disease [3], and medical treatment [4, 5].

Frequently used methods for cell lysis are based on mechanical [6, 7, 8], physical [9, 10], chemical [11], or biological principles [12]. For instance, cell walls are disrupted via repeated freeze/thaw cycles, by heating of the cells to temperatures above 60 °C or by osmotic pressure. Alternatively, cells are lysed via bead milling, sonication,

or lyophilization. Biological lysis methods often use the enzyme lysozyme or bacteriophages, and chemical methods utilize chaotropic agents at high concentrations. Depending on the microorganism, and on the availability of equipment, all of these procedures are currently used in research and industrial laboratories. Main disadvantages of these procedures are the labor intensity, time requirement, damage to nucleic acid and proteins, and also adulteration of the cell lysate with other compounds, which have to be separated from molecules of interest (such as nucleic acids and proteins).

A rapid, "clean", non-diluting and non-damaging cell lysis method would greatly ameliorate the situation of cell sample preparation. Therefore, experiments for laser-induced lysis of microorganisms was investigated. Laser microsurgery has demonstrated that cell membranes could be locally destroyed, while keeping the interior of the cell undamaged [13, 14, 15, 16, 17, 18]. Wavelengths above 750 nm are typically used in such experiments. Other experiments have been described in which a shock wave is generated in a solution in which cells or cellular components are positioned. Cells were lysed in milliseconds or less and intracellular components were analyzed subsequently by suction into a capillary [19].

Applying similar principles, we describe in this paper the development of a laser-induced cell lysis system, which rapidly and effectively disrupts cell membranes without damaging intracellular molecules. *E. coli* cells were used as model analytes, but optimized protocols were also applied to other microorganisms. To avoid ambiguity, samples of low cell concentrations were investigated (i.e., at maximum 2000 cells per sample, and in most experiments fewer than 200 cells per sample). Low continuous power lasers were applied to minimize damaging effects of intracellular molecules due to laser treatment. At wavelengths above 500 nm, RNA release and potential protein damage were investigated.

Materials and methods

Materials. Microorganisms were obtained from Dr. Randy Worobo, Cornell University, Geneva, NY. General chemicals and growth media were obtained from VWR Scientific, Rochester, NY, and Sigma, St. Louis, MO. RNA extraction and NASBA reagents were obtained from Qiagen, Valencia, CA. The *E. coli* biosensor was provided by Richard N. Cohen, Cornell University, Ithaca, NY. Lasers used were: continuous-wave argon-ion (514 nm), titanium-doped sapphire (800 nm), and Nd:YAG (1064 nm) lasers were used, along with femtosecond-pulse chromium-doped forsterite (1250 nm) and erbium-doped fiber (1550 nm) lasers. Although the last two lasers are pulsed, only the average powers matter in these experiments. A laser pointer (564 nm, 5 mW) was obtained in a local store.

Preparation of bacteria cultures and samples. All strains used in this study were provided by Dr. Randy Worobo, Cornell University, Geneva, NY, and were routinely grown on 3% Tryptic Soy broth (TSB) or agar at 37°C (*S. cerevisiae* at 30 °C), as appropriate. Cells in growth phase were chosen as samples. Initially, the cell concentration was determined by correlating the optical density (OD) measured at 600 nm with the number of colonies formed on TSB agar plates after overnight incubation. Typically, an OD value of 0.03 to 0.06 correlated to 25,000–50,000 cells per μL of sample. Samples were diluted in TSB to obtain concentrations of 25–150 cells/μL. Each experiment was carried out with six to eight replicates. In addition, six to eight controls were used for each set of experiment to obtain the exact concentration of cells in the samples. For the laser treatment, 2 μL of sample were placed onto a hydrophobic surface (such as parafilm) and exposed to the lasers for 4 min. In order to position the [2 μL] samples in an infrared laser beam, an IR card wrapped in parafilm film was used, which assists in viewing the IR beam.

Each set of laser-exposure experiments consisted of six to eight samples, and six to eight negative controls (i.e., non-treated cells). The [2 μL] samples were diluted with 18 μL of 3% TSB and plated on TSB agar plates, incubated overnight at 37 °C (or 30 °C for *S. cerevisiae*). Colonies formed were counted and the percent laser-induced lysis calculated by correlating the number of colonies of laser-treated samples with those of the negative controls.

As positive controls, initial experiments were carried out using one to three positive controls (sonication of 200 μL of sample for 45 s at 20 W), which in all cases resulted in complete lysis of the *E. coli* cells.

Determination of enzyme activity. Horseradish peroxidase (HRP) was chosen as enzyme marker serving as a proof of principle. Since a pure enzyme solution was elected for these assays, a stable enzyme was used to determine, if these would be affected by laser treatment. Future studies need to include more labile proteins. The HRP activity was assayed as previously described [20, 21]. In brief, 0.25 U/mL of horseradish peroxidase in water were added to a mixture of 1.5 mL 1.7 mmol/L H_2O_2 in 0.2 mol/L phosphate buffer, pH 7.0, and 1.4 mL 2.5 mmol/L 4-aminoantipyrine with 0.17 mol/L phenol. The reaction was monitored at a wavelength of 510 nm for 4–5 min. As a negative control (i.e., denatured protein), the enzyme solution was incubated at 90 °C for 10 min prior to determining the catalytic activity.

Experimental set up for laser exposure of cell/protein solution. The experimental setup for laser exposure of samples is shown in Fig. 1. The laser beam is reflected using flat silver mirrors to obtain vertical exposure of the sample. The beam is focused using a focusing lens just above the sample (focal length: 75.6 mm) and is about 2 mm in diameter. The sample itself was placed on an infrared card, which is wrapped using a single layer of parafilm. The parafilm provides a hydrophobic surface on which aqueous samples form a sphere with least contact area to the parafilm surface. This ensures consistency of sample dimensions throughout the experiments and minimizes loss of cells due to nonspecific binding to the parafilm surface.

Determination of laser effect on cells and enzymes. As described above, cell lysis was determined as survival rate of the microorganisms after laser exposure, (i.e., cells damaged by laser treatment are unable to grow to colonies when plated onto TSB agar plates). Percent cell lysis was determined by correlating the number of colonies resulting from treated samples to the number of colonies resulting from untreated samples. Enzyme damage was calculated by comparing the catalytic activity of horseradish peroxidase in untreated samples to the enzymatic activity of laser-treated enzyme solutions.

Determination of RNA release. RNA release from laser treated *E. coli* cell samples was determined by the detection of one specific mRNA sequence. Protocols for the detection of the mRNA sequence were published previously [22, 23]. In brief, the heat shock protein hsp70 mRNA was detected by first inducing the cells to produce the mRNA, and then subsequently lysing the cells (using sonication or laser treatment). The specific hsp70 mRNA was amplified using RNampliFire (Qiagen). Finally, amplified RNA was detected using agarose gel electrophoresis and an *E. coli* specific biosensor. As shown before, mRNA from as few as 40 *E. coli* cells can be detected using this method. The biosensor assay is based on the hybridization of amplified RNA molecules with two sets of