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Research Article

Extraction of nucleic acids from bacterial spores using bead-based mechanical lysis on a plastic chip

This paper describes an experimentally simple and efficient way of integrating bead-based mechanical cell lysis on a plastic chip. The chip is fabricated from machined slides of poly(methylmethacrylate) and accommodates a metal disk along with solid microbeads in a designated lysis chamber. Magnetic actuation of the metal disk induces collisions and frictional forces within the lysis matrix causing cell disruption. The efficiency of nucleic acid extraction was investigated using spores of *Bacillus atrophaeus* subsp. *globigii* and the amount of genomic DNA in the lysates has been quantified by real-time PCR. Compared to a standardized DNA extraction method based on BD GeneOhm™ Lysis Kit, the yield was dependent on the composition of the lysis matrix, including size and relative amount of microbeads, along with instrumental parameters, such as duration and frequency of agitation. The interplay of these parameters should allow for optimizing lysis protocols to faithfully disrupt any particular type of cell without affecting the genetic material to be extracted.

Keywords: Bacterial spores / DNA extraction / Mechanical cell lysis / Microfluidics / PCR

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1 Introduction

The extraction of nucleic acids marks a key step in the process of detecting microbial species using PCR or other nucleic acid testing techniques [1]. To extract DNA (and other molecules of interest, such as RNA or proteins), cells of a particular sample must be subjected to a lysis process during which the cell envelope is disrupted and intracellular content released in the surrounding medium. There are a number of methods using chemical or physical principles that can be used to this end, many of which are being investigated for automation and integration in lab-on-a-chip devices [2–15]. Chemical methods rely on the presence of lysing agents, such as organic solvents, detergents or enzymes that degrade the surrounding layers of a cell. Despite the fact that chemical cell disruption has been shown to be compatible with the processing of small-scale sample volumes on a fluidic chip [3–5], lysis agents generally need to be removed from the analyte to prevent interference with subsequent attempts to amplify and detect nucleic acids,

which in turn can lead to relatively complex chip configurations [5]. Moreover, chemical methods encounter limitations for cells that are encapsulated by sturdy and resistant envelopes, such as plant, fungi and spores. Physical methods, on the other hand, are relatively general with respect to sample processing and scope of application, but often demand for additional instrumentation to be implemented on or in close proximity to the chip. A common approach relies on exposure to low- or high-frequency ultrasound for shock waves dissipating in the liquid medium cause cavitation and breakdown of the cellular structures. Sonication methods can be readily applied to a broad range of sample volumes, effectively promoting microfluidic integration of sample preparation protocols [6–8]. On-chip cell disruption has further been shown in conjunction with thermal [9], electrochemical [10] and hydrodynamic (shear-force) methods [11, 12]. Bead-based mechanical lysis employs collisions and frictional forces between abrasive particles (e.g. glass, ceramic or metal) that are agitated together with a cell suspension. Agitation is realized, for example, by shaking, stirring or acoustic actuation of the lysis matrix. Although the presence of beads limits the potential for miniaturization to some extent, reliability and ease of implementation render bead-beating methods attractive for use in automated sample preparation systems [13–15].

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Abbreviation: PMMA, poly(methylmethacrylate)

Herein, we demonstrate how to integrate bead-based mechanical cell lysis on a plastic chip in a convenient and experimentally simple fashion. Agitation of the lysis matrix is realized through the displacement of a metal disk inside a designated lysis chamber using an oscillating magnetic field. Previously, this concept has been elegantly applied by Madou and co-workers in their development of a compact disk for sample preparation [14, 15]. As opposed to a spinning disk-like device, we target for a chip that remains stationary, potentially increasing the level of safety during operation. In essence, we investigated the extraction of genomic DNA from bacterial spores of *Bacillus atrophaeus* subsp. *globigii* [16] (herein referred to as *B. atrophaeus*). As reproductive structures adapted for survival in unfavorable conditions, spores are generally more difficult to disrupt than vegetative bacterial cells or other microbial organisms, which renders them ideal candidates for evaluating the performance of a lysis process. Moreover, *B. atrophaeus* qualifies as surrogate for *Bacillus anthracis*, which has been classified by the Centers for Disease Control and Prevention as a high-priority biological agent regarding its ability to spread, the severity of disease it may induce and its anticipated impact on public health [17]. In fact, the median lethal dose (LD50) of aerosolized *B. anthracis* strain Ames has been evaluated in Rhesus Macaque at 5.5×10^4 bacteria [18]. In the present study, we performed cell lysis with 10^4 spores of *B. atrophaeus*, and investigated how the performance of this process is affected by the nature and the size of the microbeads, the solid mass fraction in the lysis matrix as well as the time and the frequency of agitation. We monitored the concentration of extracted DNA using real-time PCR and compared our results to those obtained with a commercial cell lysis kit serving as a reference.

2 Materials and methods

2.1 Set-up

The stage ($30 \times 30 \text{ cm}^2$ in area, 2.9 cm in height) and the rotating bar (20 cm in length) were custom-machined from polycarbonate (Lexan, SABIC Innovative Plastics, Pittsfield, MA). Aluminum construction rails from Thorlabs (Newton, NJ) were used as support structures. Permanent magnets (nickel-plated neodymium blocks, 12.7 mm in width, length and height) were purchased from K & J Magnetics (Jamison, PA). Each magnet comprised a through-hole oriented perpendicular to the magnetization direction, which was used for attachment. The electromotor (Anaheim Automation, Anaheim, CA) driving the rotating bar had an output power of 30 W and supported a maximum speed of 4000 rpm. The motor was connected to a power supply and a sampling oscilloscope (Tektronix, Beaverton, OR) to monitor the frequency of rotation.

2.2 Preparation of chips

Poly(methylmethacrylate) (PMMA) slides ($25 \times 75 \text{ mm}^2$ in area, 1 mm in thickness) were prepared by injection molding

of Plexiglas® VS UVT (Altuglas International, Philadelphia, PA) using a Boy 30A injection tool (Dr. Boy, Neustadt-Ferthal, Germany) that was operated at a temperature of 220–240°C, an injection speed of 30 mm/s, and a pressure of 132 bar. The mold (stainless steel, custom-fabricated) was cooled for 15 s before slides were released. The lysis chamber was machined with a 9.5 mm (diameter) end mill using a Sherline 2000 vertical milling machine (Sherline Products, Vista, CA) that was operated by a computerized numerical control system (Flashcut, Deerfield, IL). The length of the cut-out section was maintained at 15 mm (excluding the regions of curvature). Access holes were drilled using a 1.6 mm (diameter) drill bit. During the assembly of the slides, a metal disk (5 mm in diameter and 0.5 mm in height, V & P Scientific, San Diego, CA) was inserted into the lysis chamber. The stack was supported with standard microscope glass slides (Fisher Scientific, Ottawa, ON) and sealed using a FoodSaver® Vacuum Packaging System (Brampton, ON), followed by exposure to 85°C at 90 psi for 30 min using a BAC-23 pressure tool (Baron Autoclave, Santa Fe Springs, CA) to effectuate bonding of the PMMA slides. Silica and zirconia beads were purchased from OPS Diagnostics (Lebanon, NJ). Beads were washed with ethanol (Commercial Alcohols, Brampton, ON), 0.1 M HCl (Sigma-Aldrich Canada, Oakville, ON) and deionized water ($18.2 \text{ M}\Omega \text{ cm}$, Millipore, Billerica, MA) followed by drying at 80°C for 12 h. Proper amounts of beads were transferred manually and in dry state into the lysis chamber using a refined micropipette tip (VWR International, West Chester, PA).

2.3 Bacterial spores

Spores of *B. atrophaeus* subsp. *globigii* strain CCRI-9827 (Collection du Centre de recherche en infectiologie, Québec, QC) were prepared, purified and stored according to published procedures [16, 19]. For cell lysis experiments, the suspension was diluted with TE buffer solution (10 mM Tris, 1 mM EDTA, pH 8.0, Sigma-Aldrich) to a concentration of 100 spores/ μL . Suspensions containing 50 spores/ μL have also been tested. Fluorescence labelling was done by incubating spores at 0.5 McFarland with 0.02% FITC in 0.5 M sodium carbonate buffer (made by combining 0.5 M Na_2CO_3 and 0.5 M NaHCO_3 to obtain pH 9.5, Sigma-Aldrich) for 45 min with gentle agitation followed by washing twice with a solution of PBS (137 mM NaCl, 6.4 mM Na_2HPO_4 , 2.7 mM KCl, 0.88 mM KH_2PO_4 , pH 7.4, Sigma-Aldrich).

2.4 DNA extraction

In a typical experiment, 100 μL of the diluted suspension (i.e. 10^4 spores) were transferred to the lysis chamber using a micropipette. The access holes were sealed using adhesive polyester film (designed for PCR plates, 125 μm in thickness, VWR International) before the chip was placed on the sample platform to proceed with the lysis process. Upon completion, the lysate was recovered and transferred into a 1.5 mL tube (Eppendorf Canada, Mississauga, ON) followed by heating to

95°C for 2 min. BD GeneOhm™ Lysis Kit (BD Diagnostics, Québec, QC) was used according to the recommendations from the supplier [20] and provided a reference for each set of experiments. Specifically, 100 µL of the diluted spore suspension were transferred into a designated lysis tube followed by agitation on a vortex Genie® 2 (Scientific Industries, Bohemia, NY) at the maximum adjustable speed for a period of 5 min, which was complemented by a heating step at 95°C for 2 min. Highly purified genomic DNA serving as a PCR reference sample was prepared from mid-log-phase cells of *B. atrophaeus* strain CCRI-9827 using MagneSil KF isolation kit (Promega, Madison, WI) on a KingFisher ML instrument (Thermo Fisher Scientific, Waltham, MA).

2.5 PCR

A fragment of the *atpD* gene of *B. atrophaeus* was amplified using two specific primers (5'-CAC TTC ATT TAG GCG ACG ATA CT-3' and 5'-TTG TCT GTG AAT-CGG ATC TTT CTC-3') along with a 5'-FAM-labeled TaqMan probe (5'-CGT CCC AAT GTT ACA TTA CCA ACC GGC ACT GAA ATA GG-3') [16]. PCR mixtures contained 0.4 µM of each primer (Integrated DNA Technologies, Coralville, IA), 0.1 µM of the TaqMan probe (Biosearch Technologies, Novato, CA), 0.025 units/µL of Taq DNA polymerase (EconoTaq, Lucigen, Middleton, WI), 3.45 mM MgCl₂ (Thermo Fisher Scientific),

200 µM dNTP (Invitrogen Canada, Burlington, ON), 10 mM Tris-HCl (pH 9.1, Sigma-Aldrich), 50 mM KCl (Sigma-Aldrich), 3.3 mg/mL BSA (Sigma-Aldrich) and 0.1% Triton X-100 (Sigma-Aldrich) along with 2 µL of the analyte. Each DNA extract was analyzed in duplicates. Real-time PCR measurements were performed using LightCycler® 1.5 (Roche Diagnostics, Basel, Switzerland) or Rotor-Gene (Corbett Research, Sydney, Australia) thermocycling instruments. The amplification process involved an initial heating step at 95°C for 3 min, 45 cycles of three steps including 5 s at 95°C, 15 s at 60°C and 20 s at 72°C. The concentration of DNA present in the analyte was quantified using reference samples containing 50, 100, 150, 200 and 250 copies/µL of highly purified genomic DNA in TE buffer solution, which provided calibration points for each series of amplification reactions. Negative controls containing no added DNA were routinely included in each set of PCR measurements.

2.6 Imaging of spores

Fluorescence imaging of *B. atrophaeus* spores labeled with an FITC marker ($\lambda_{\text{ex}} = 495 \text{ nm}$; $\lambda_{\text{em}} = 521 \text{ nm}$) was done using an Olympus FV300 confocal microscope (Olympus Canada, Markham, ON) equipped with an argon-ion laser for excitation at 488 nm. Suspensions of treated and untreated spores were diluted 1:1 v/v with a warm solution of 1%

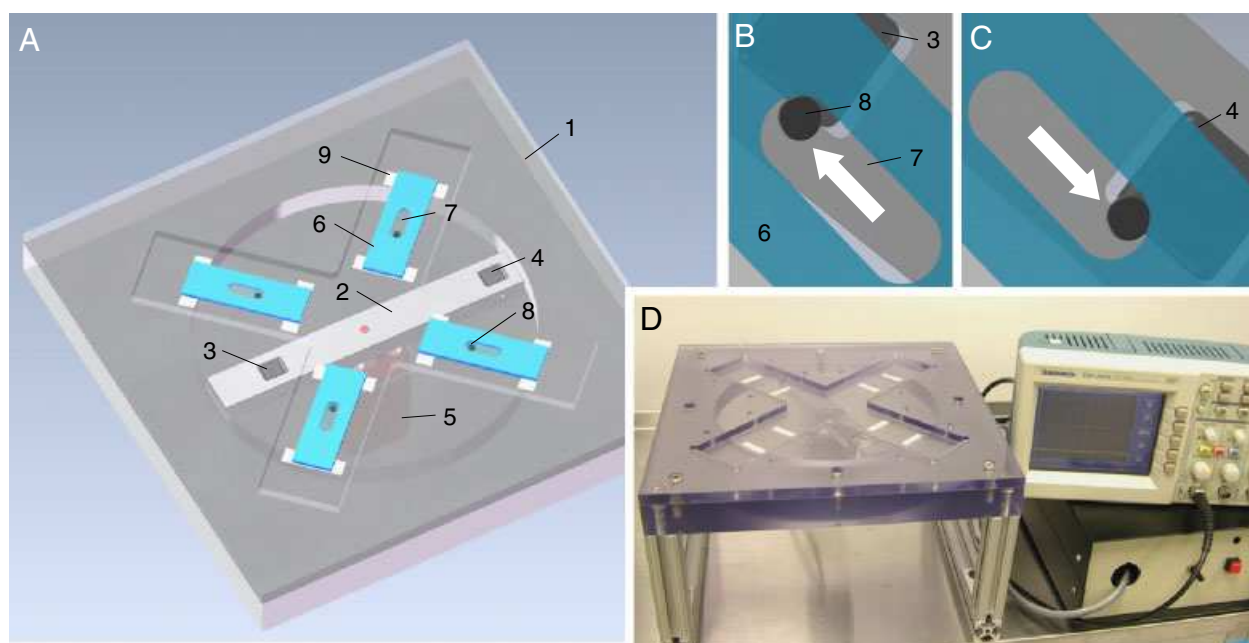


Figure 1. Experimental setup used for mechanical cell disruption. (A) Three-dimensional view of the stage (1) and the rotating bar (2) which moves in-plane below the surface. The rotating bar is equipped with two permanent magnets (3, 4) that are arranged in an asymmetric fashion (e.g. with a shift of 15 mm along the long axis of the bar). The rotating bar is connected to an electromotor (5) located further below the stage. The setup can accommodate up to four chips (6), each of which contains a lysis chamber (7) and a metal disk (8) at its interior. The chips are kept in designated sections using a double-sided adhesive film (9). (B and C) The metal disk on the chip is displaced via an oscillating magnetic field induced by the rotational movement of the bar. The arrow in each image indicates the respective direction of the metal disk moving across the lysis chamber. (D) Photograph of the setup as it was used in this study, depicting the lysis platform (left-hand side) along with the respective control units (right-hand side).

agarose (VWR International) in PBS and sandwiched between a glass microscope slide and a cover slip prior to inspection. Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, available at <http://rsbweb.nih.gov/ij/>).

3 Results and discussion

3.1 Setup

The instrumental setup comprises a sample stage, a rotating bar and an electrical motor as the principal components, whose arrangement is illustrated in Fig. 1A. The stage serves as a support for the lysis chips, each of which is placed in a

designated cavity. The current design allows for processing four chips simultaneously, yet it would equally be possible to include a larger number of cavities (i.e. 8–10) on the sample stage. The rotating bar moves in-plane underneath the surface and is connected to a motor located further below the stage. The rotating bar comprises two permanent magnets that are fixed at opposite positions in an asymmetric manner with respect to the lysis chamber. This configuration causes the magnetic field to oscillate between the two ends of the lysis chamber when passed by the magnets during rotation. As a consequence, the metal disk is displaced from one end of the lysis chamber to the other, as illustrated in Figs. 1B and C. The moving disk induces agitation of the beads, which in turn promotes lysis of the spores being present in the chamber. We monitored the speed of the rotating bar using an oscilloscope (Fig. 1D), allowing for adjustment and read-out of the frequency at any time during an experiment. Control measurements confirmed agreement between the frequency of the rotating bar and the displacement of the metal disk in the lysis chamber, up to a velocity of at least 600 rpm (data not shown).

3.2 Lysis chip

We devised a relatively simple chip to test the functioning of the setup and evaluate the performance of the lysis process (Fig. 2). The chip is fabricated from slides of PMMA and comprises an embedded lysis chamber with a magnetizable metal disk at its interior. We selected PMMA since it provides a number of advantages for the fabrication and handling of the chips. First, it is commercially available in large quantity at a moderate price. Second, it is possible to shape a desired piece using molding or embossing techniques, which can be complemented by drilling, milling or polishing operations. Third, multiple sheets of PMMA can be bonded together through thermal treatment. Fourth, the optical transparency of this plastic material makes it suitable for applications that would require observation inside the chip. Finally, PMMA has been shown to be compatible with procedures that involve nucleic acids, including extraction, electrophoretic separation and amplification using PCR [21]. Figure 2A illustrates the different components that form a single chip, which includes a bottom slide, at least one center slide

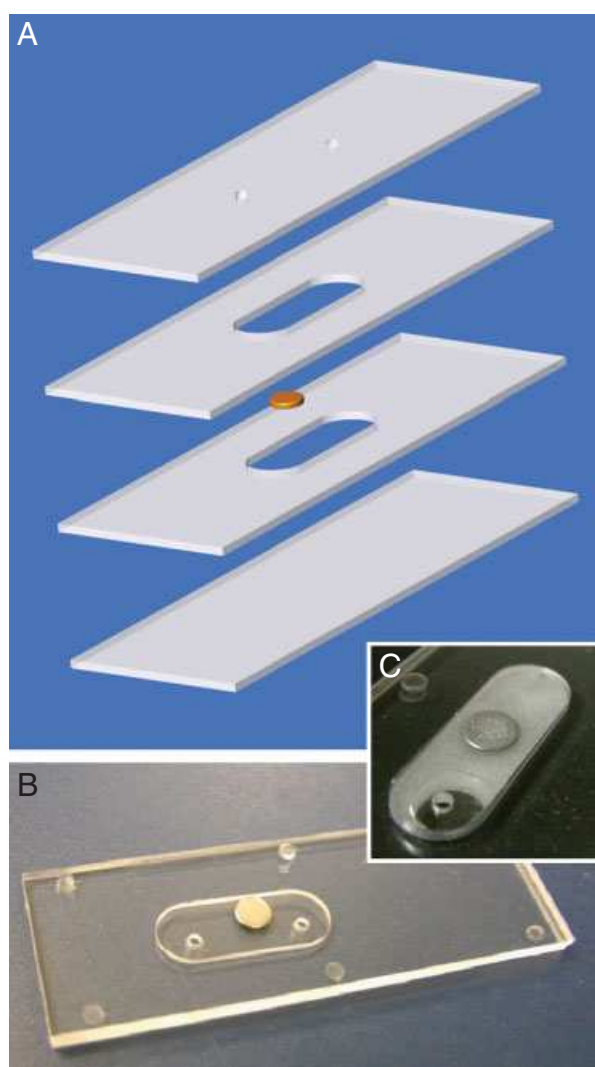


Figure 2. Design and fabrication of the lysis chip. (A) A set of PMMA slides is superimposed and bonded through thermal treatment, enclosing a metallic disk inside the lysis chamber. (B) Photograph of a lysis chip after assembly and bonding. (C) Close-up view of a lysis chamber containing silica beads of 100 μm in diameter and a suspension of bacterial spores.

Table 1. Microbeads and their use within this study

Material	Nominal size (μm)	Measured size ^{a)} (μm)	Employed as
Silica	100	$106 \pm 17^{\text{b}}$	Pure batch and mixtures
	400	$456 \pm 34^{\text{c}}$	Pure batch and mixtures
Zirconia	100	$123 \pm 20^{\text{b}}$	Pure batch
	200	$330 \pm 21^{\text{c}}$	Pure batch

^{a)} As determined by using optical microscopy.

^{b)} Average diameter and standard deviation were calculated on the basis of 20 beads selected in a random fashion.

^{c)} Average diameter and standard deviation were calculated on the basis of 15 beads selected in a random fashion.

containing the lysis chamber as a rounded cut-out section and a top slide with two circular openings providing access to the chamber underneath. A metal disk is inserted in the cut-out area, followed by bonding of the slides at elevated temperature and pressure. An example of the assembled chip is shown in Fig. 2B. We mostly produced chips with two center slides, yielding a nominal capacity of 426 μL for the respective lysis chamber. This configuration allowed for processing a sample volume of 100 μL (or more), while providing the possibility of changing bead-to-volume ratios in the lysis matrix. Bonding of the plastic slides was leak-proof as illustrated by the example in Fig. 2C. The dimensions of the chamber can be increased further to accommodate larger volumes, if necessary.

3.3 Effect of matrix composition

In an attempt to determine optimal lysis conditions, we agitated a suspension of spores with silica or zirconia beads for 5 min, and varied the solid mass fraction of the lysis matrix. We selected BD GeneOhm™ Lysis Kit as a benchmark process since it effectively promotes cell lysis (i.e. with 98.8% yield or more) for a number of samples, including spores of *B. anthracis* and *Bacillus subtilis* as evaluated by PCR amplification of genomic DNA in lysates [16, 20, 22]. Compared to other relatively fast extraction methods, this kit has further been shown to provide the highest yield of DNA from *Mycobacterium tuberculosis* [23]. As indicated in Table 1, beads of the same nominal size are not monodispersed but rather display variable diameters, which, in principle, should help

diminish void spaces and improve the number of inter-particle collisions. The findings presented in Fig. 3 suggest that the concentration of DNA increases with the bead-to-volume ratio until reaching a maximum at around 1.5–2.0 mg/ μL . The disk alone with no beads added to the spore suspension (e.g. 0 mg/ μL) did not release any traces of DNA detectable by PCR. At relatively low ratios (e.g. <1.0 mg/ μL), the disk induces vigorous agitation of the lysis matrix, yet the number of collisions between beads remains insufficient for effectively promoting cell lysis. The ratios located around the maximum of each curve (e.g. 1.5–2.0 mg/ μL for 100 μm beads) are favorable for cell disruption, even though the yield obtained under any of these conditions remains inferior to that achieved by the BD GeneOhm™ Lysis Kit. The highest yield was 68% on average, and was obtained with zirconia beads of 100 μm in diameter at 2.0 mg/ μL , although this value does not differ significantly with respect to adjacent data in the graph. Interestingly, the yield tends to decline when the amount of beads becomes too high (i.e. starting at around 2.0 mg/ μL for silica and 2.5 mg/ μL for 100 μm zirconia beads). We noticed that beads accumulate at the extremities of the lysis chamber at 2.5 mg/ μL , thereby compromising the mobility of the disk (at least when a frequency of 250 rpm is used for agitation), which may explain this finding to some extent. We observed a significant drop in performance when zirconia beads with a nominal diameter of 200 μm were employed, suggesting that larger particles alone are less suitable for the disruption of bacterial spores. This finding is consistent with an increase in void spaces between particles that are unproductive for cell lysis [22].

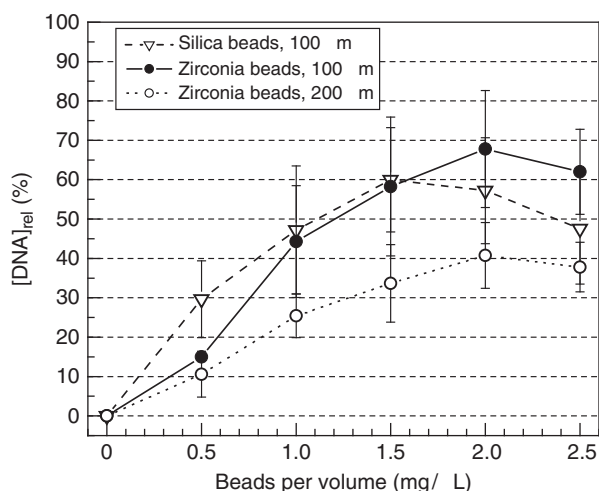


Figure 3. Plot of the DNA concentration extracted from spores of *B. atrophaeus* as a function of solid mass fraction using silica and zirconia beads that are 100 and 200 μm in diameter, respectively. Experiments were carried out at a rotational speed of 250 rpm over a period of 5 min. Values of concentration were determined using PCR and are presented relative to the average yield of a standardized DNA extraction method using BD GeneOhm™ Lysis Kit. The lines in the graph serve as a guide to the eyes. Standard errors are derived from one to five samples that were quantified in duplicates.

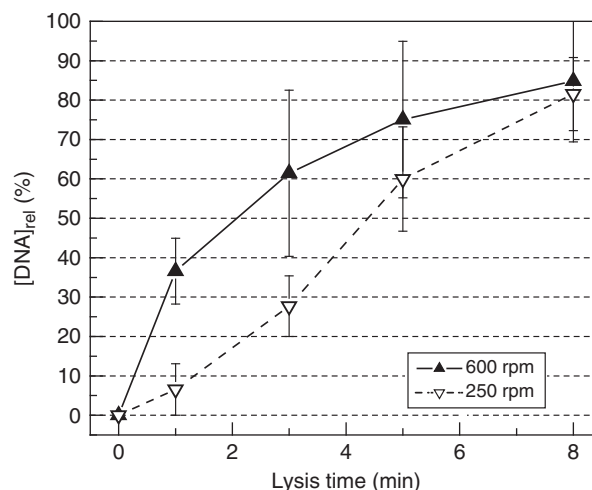


Figure 4. Plot of the DNA concentration extracted from *B. atrophaeus* as a function of time and frequency of agitation. Experiments were carried out using silica beads (100 μm in diameter) with a solid mass fraction of 1.5 mg/ μL . Values of concentration were determined using PCR and are presented relative to the average yield of a standardized DNA extraction method using BD GeneOhm™ Lysis Kit. The lines in the graph serve as a guide to the eyes. Standard errors are derived from one to five samples that were quantified in duplicates.

3.4 Settings

We used silica beads (100 μm in diameter) as a model system to assess the influence of both time and frequency of agitation on the efficiency of the lysis process. Figure 4 compares the relative concentration of DNA when we agitated the lysis matrix at 250 and 600 rpm for up to 8 min. It is apparent from the graph that the concentration of traceable DNA molecules increases progressively with the lysis time, reaching its maximum beyond the time scale being recorded here. A higher

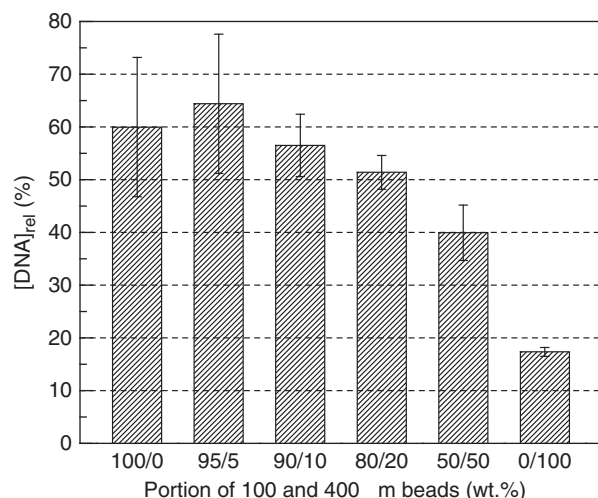


Figure 5. Plot of the DNA concentration extracted from *B. atrophaeus* as a function of mixing ratio for silica beads that are 100 and 400 μm in diameter. Experiments were carried out using a solid mass fraction of 1.5 mg/ μL and a rotational speed of 250 rpm applied for a period of 5 min. Values of concentration were determined using PCR and are presented relative to the average yield of a standardized DNA extraction method using BD GeneOhm™ Lysis Kit. Standard errors are derived from one to five samples that were quantified in duplicates.

rotational speed noticeably improved the yield for shorter periods of agitation (i.e. 1 and 3 min). However, the difference in efficiency becomes less pronounced for 5 min, while the concentration of extracted nucleic acids resulting from these two experiments became largely comparable at 8 min (i.e. 82 versus 85% for 250 and 600 rpm, respectively). It is reasonable to assume that the gain in lysis efficiency tends to decrease for processing times longer than 8 min. A higher frequency of agitation generally induces a larger number of collisions in the lysis matrix making it possible to disrupt bacterial spores in a timely fashion. In the context of a real-case scenario, the rapidity of sample preparation and detection may be more important than their sensitivity since each minute counts.

3.5 Bead mixtures

Ménard and Picard emphasized the possibility of using mixtures that comprise both smaller and larger particles in the lysis matrix to improve the efficiency of cell disruption [22]. When agitated, bigger particles can contribute effectively to the motion of their smaller counterparts, thereby increasing the probability of inter-particle collisions. We verified this option by producing binary mixtures of silica beads that were 100 and 400 μm in diameter to constitute the lysis matrix. The graph in Fig. 5 illustrates how the mixing ratio affects the concentration of extracted DNA. The data suggest no significant improvement in efficiency, even though a small amount (i.e. 5%) of 400 μm beads added to the matrix tends to provide the best results in this series (i.e. 65 versus 60%). Any larger percentage of 400 μm beads diminished the lysis efficiency until an 18% yield was finally obtained for the pure batch. This is consistent with the findings presented in Fig. 3, where 100 μm beads lead to higher DNA concentrations than 200 μm beads. We believe that it may be possible to achieve better performances by mixing beads with a greater diversity in size. Preceding work has shown, for example, that effective cell disruption can be

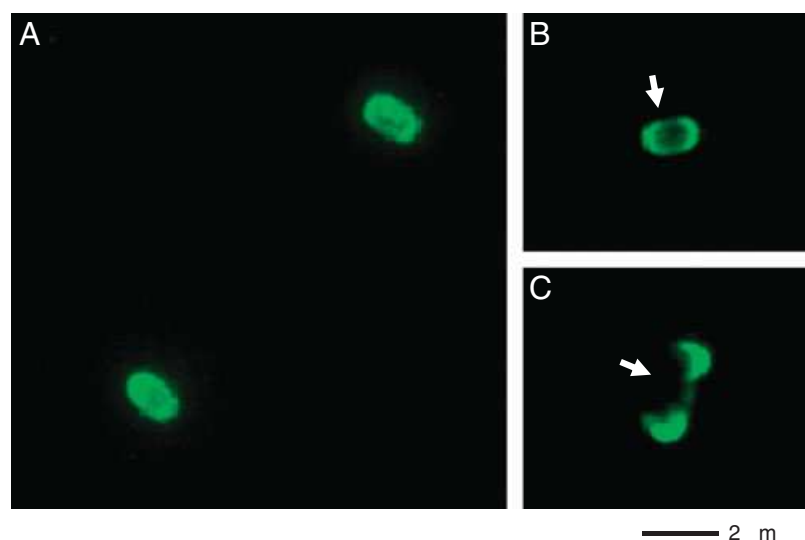


Figure 6. Fluorescence micrographs showing FITC-labeled spores of *B. atrophaeus* immobilized on agarose gel. (A) Intact spores prior to treatment. (B and C) Selected examples of spores illustrating the effect of cell lysis upon agitation with silica beads (100 μm in diameter) at a solid mass fraction of 2.1 mg/ μL using a rotational speed of 250 rpm for 5 min. The arrows indicate the locations that were visibly affected by the treatment.

achieved when glass beads ranging from 150–212 and 710–1180 µm in diameter provide the lysis matrix [22]. However, a more detailed investigation of this option was beyond the scope of this study.

3.6 Fluorescence imaging of spores

In addition to PCR measurements, we performed confocal microscopy imaging to visualize FITC-labeled spores in 3-D and monitor any possible changes in cell morphology associated with the lysis process (Fig. 6). Untreated, spores are of elliptical shape with dimensions ranging from 0.9–1.1 and 1.4–1.6 µm in width and length, respectively (Fig. 6A). Moreover, they display homogeneous fluorescence at the spore coat, which is well observed within the agarose gel used for immobilization. The differences between treated and untreated samples were subtle as spores mostly retained their initial size and shape upon agitation for a broad range of experimental conditions. We found, however, that treated spores commonly exhibit non-homogeneous fluorescence, as shown by the example in Fig. 6B. It is reasonable to assume that this alteration is due to partial disruption of the multilayer envelope, even though the degree of damage induced by the treatment cannot be revealed unambiguously using confocal microscopy imaging. Extensive fragmentation of spores was observed (Fig. 6C) but remained an exception and hence should be considered as an extreme representation of spore lysis. These findings overall confirm the possibility of provoking cell disruption through agitation of the lysis matrix, providing access to intracellular genomic DNA that can be monitored precisely using PCR.

4 Concluding remarks

The work presented in this paper demonstrates the possibility of performing cell lysis on a plastic chip that leads to PCR-quality DNA extracted from bacterial spores. The prototype setup allows up to four lysis experiments to be completed in parallel within less than 10 min. Even though the prototype instrument is relatively spacious, each of its components (including driving and control units) can be reduced in size, potentially promoting the development of an integrated lab-on-a-chip platform. The plastic chip remained at a rather low level of complexity, yet it should be possible to include other functional elements in the design that would allow for sample collection or downstream processing of the lysate. While spores of *B. atrophaeus* constitute a convenient model system for this study, we believe that the method should be suitable for disrupting a broad range of other microorganisms without the need for drastic alteration of the experimental settings. The amount of bacterial spores tested with this system (i.e. 10^4 spores of *B. atrophaeus*) is more than five times lower than LD50 of *B. anthracis* in Rhesus Macaque. The ability to extract nucleic acids from biological agents within minutes on the field should further help resolving complex issues of public

safety and medical management in the event of a bioterrorist attack.

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