Analytical Biochemistry 510 (2016) 33–40

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Microwave-accelerated method for ultra-rapid extraction of *Neisseria gonorrhoeae* DNA for downstream detection

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ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form
8 June 2016
Accepted 9 June 2016
Available online 17 June 2016

Keywords:
Neisseria gonorrhoeae
Gonorrhea
Microwaves
DNA extraction
DNA fragmentation
Microwave-accelerated metal-enhanced fluorescence

ABSTRACT

Nucleic acid-based detection of gonorrhea infections typically require a two-step process involving isolation of the nucleic acid, followed by detection of the genomic target often involving polymerase chain reaction (PCR)-based approaches. In an effort to improve on current detection approaches, we have developed a unique two-step microwave-accelerated approach for rapid extraction and detection of *Neisseria gonorrhoeae* (gonorrhea, GC) DNA. Our approach is based on the use of highly focused microwave radiation to rapidly lyse bacterial cells, release, and subsequently fragment microbial DNA. The DNA target is then detected by a process known as microwave-accelerated metal-enhanced fluorescence (MAMEF), an ultra-sensitive direct DNA detection analytical technique. In the current study, we show that highly focused microwaves at 2.45 GHz, using 12.3-mm gold film equilateral triangles, are able to rapidly lyse both bacteria cells and fragment DNA in a time- and microwave power-dependent manner. Detection of the extracted DNA can be performed by MAMEF, without the need for DNA amplification, in less than 10 min total time or by other PCR-based approaches. Collectively, the use of a microwave-accelerated method for the release and detection of DNA represents a significant step forward toward the development of a point-of-care (POC) platform for detection of gonorrhea infections.
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Gonorrhea (GC) is the second most prevalent sexually transmitted infection (STI) reported to the Centers for Disease Control and Prevention (CDC) [1]. The use of nucleic acid amplification tests (NAATs) for detection of gonorrhea has significantly increased over the past two decades primarily due to its ease of use and high sensitivity. Nevertheless, there is a continued need for the development of faster, more sensitive, and cheaper molecular approaches. Microwave-accelerated metal-enhanced fluorescence (MAMEF) is a platform that has shown significant promise as an analytical tool for the rapid and sensitive detection of bacterial pathogens [2–7]. MAMEF is an amplification-free hybridization assay that combines the benefits of metal-enhanced fluorescence to increase assay sensitivity for detection with low-power microwaves, which accelerates biological recognition events [8]. The increased sensitivity of the assay is underpinned by the plasmonic-based enhancement of fluorescence emission in the near field, resulting from the nonradiative transfer of energy from the excited fluorophore to silver nanoparticles, followed by the emission of the coupled quanta [9]. The use of low-power microwaves reduces the assay run time by up to several 1000-fold, which when combined with enhanced assay fluorescence provides for a powerful platform for both ultra-rapid and sensitive bioassays [10,11].

One of the optimal technical aspects of MAMEF is the requirement for small DNA fragments (<100 bp) for DNA hybridization. Although a variety of approaches including nebulization, mechanical and acoustic shearing, and ultrasonic baths can be used to generate DNA fragments of different sizes (100 bp to 8 kb), these approaches require sophisticated instrumentation [12]. Microwave irradiation is primarily known as a tool for sterilization purposes, but most recently it has been used for other purposes, including acceleration of chemical reactions and release of genomic DNA. Microwaves have been shown to be effective for

Abbreviations used: GC, gonorrhea; MAMEF, microwave-accelerated metal-enhanced fluorescence; PCR, polymerase chain reaction; FDTD, finite different time domain; dsDNA, double-stranded DNA; MW, molecular weight.

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http://dx.doi.org/10.1016/j.ab.2016.06.017
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the preparation of genomic DNA from a variety of biological systems, including bacteria [13,14], bacteriophage [15], and spores [6], but also for preparation of DNA for real-time polymerase chain reaction (PCR) analysis [16,17]. More recently, microwave irradiation has been used exclusively for the purpose of DNA fragmentation for various molecular approaches. Yang and Hang recently reported on the use of microwaves to generate DNA fragments for next-generation DNA applications [18]. Although successful, their microwave irradiation procedure requires a specialized instrument and is time-consuming, unlike what is proposed by our approach here.

The most common way to decrease microwave irradiation time is by increasing the power, which can result in overheating and loss of volume. Alternatively, the irradiation time can be decreased by increasing the frequency of the interaction of the microwaves with the sample. The use of bow-tie structures as nano-antennas for focusing light onto specific substrates has been reported extensively [19]. Our approach for focusing microwaves is based on the use of bow-tie structures in the form of two disjointed equilateral gold triangles deposited on a glass microscope slide. However, contrary to light-focusing antennas, these bow-tie structures are not nanometer scale, but are in fact centimeter scale, consistent with the much longer wavelength of microwaves (~12.3 cm). These bow-tie structures help to focus microwaves onto the sample, increasing water heating both within and outside the organism to be lysed, thereby increasing lysing efficiency. The use of a silicone isolator over the bow-tie structures creates a chamber (sample well) capable of holding a specific volume of sample (liquid). We previously reported on the use of these lysing chambers to generate DNA fragments for MAMEF analysis [2–7], but we never reported on the effect of microwave power and radiation time on DNA fragmentation or investigated to what degree bow-tie structures enhance bacterial cell lysing efficiency.

In the current article, the effects of various experimental parameters such as microwave power, time, and chamber size on culture survival and DNA fragmentation are described. Furthermore, we report on the effect of disjointed bow-tie structures on lysing efficiency and compare the efficiency of highly-focused microwaves lysing to conventional heating. The efficiency of the microwave lysing technology for DNA preparation and fragmentation is further highlighted through the detection of GC DNA fragments by MAMEF isolated from vaginal swabs. Overall, the detection of GC DNA is mediated by a two-step process involving rapid extraction and fragmentation of DNA by microwaves, followed by DNA detection with the MAMEF technology.

Materials and methods

Microwave-based lysing using bow-tie geometries

Gold bow-tie geometries (Fig. 1A), which highly focus microwaves at 2.45 GHz onto samples, have been developed previously and the rationale for their use has been reported previously [20]. Our sample chambers have been theoretically designed and modeled using numerical simulations (finite different time domain, FDTD) [21] to determine the spatial and temporal profiles of the focused microwaves to optimize the heating/volume effects. The optimization of heating effects by microwave-focusing bow-tie geometries allows for the use of low-cost (~$40) commercial, low-power microwave ovens for lysing with only a few slight modifications inside, including removal of the rotating plate and insertion of a mounting device for sample holding (Fig. 2). The rapid heating of the water by microwaves (both around and within the organism) rapidly disrupts cellular membranes, resulting in the release and fragmentation of genomic material [6,7].

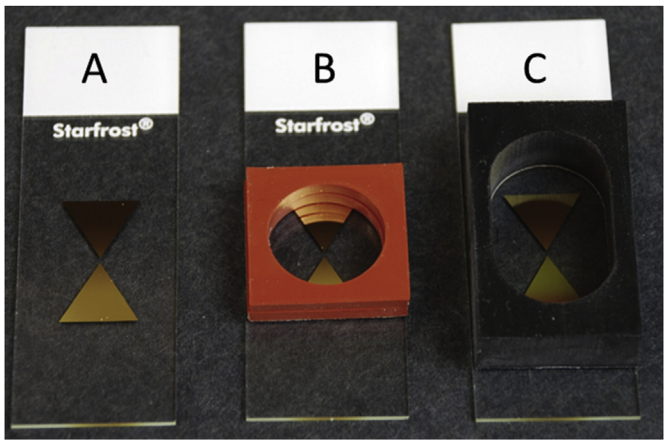

Fig.1. (A) Bow-tie disjointed gold triangles deposited on glass slides. (B) Lysing chamber with small isolator (500 μl–1 ml). (C) Lysing chamber with large isolator (up to 2 ml volume).

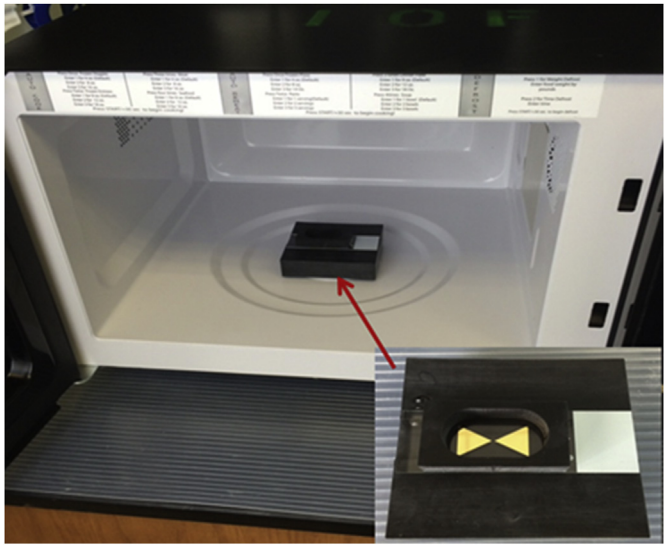

Fig.2. Household microwave fitted with a mounting device designed to hold the lysing chamber as shown in the inset.

Deposition of gold triangles on glass substrates on lysing chambers

Equilateral gold (99.999%) triangles of 12.3 mm and approximately 100 nm thicknesses (Fig. 1A) were deposited onto glass microscope slides using a BOC Edwards 306 vacuum deposition unit at a rate of 0.1 nm/s. Following the deposition of gold triangles in a bow-tie structure configuration, self-adhesive isolators were placed over the triangles to create a lysing chamber. Briefly, two layers of silicon isolators with a diameter of 20 mm were placed on top of the bow-tie region to create a chamber for lysing sample volumes from 500 μl to 1 ml (Fig. 1B). For lysing larger volumes (up to 2 ml), a single black silicon isolator (diameter = 32 mm) was used to create a lysing chamber (Fig. 1C).

Lysis of N. gonorrhoeae by conventional heating

Neisseria gonorrhoeae (ATCC 43069) was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Bacterial dilutions (10^8 to 10^4 CFU/ml) were prepared from overnight cultures in distilled autoclaved water and submitted to lysing by

conventional heating and microwaves as described below. Microbial cells were lysed by heating 4 ml of bacterial suspensions (10^8 CFU/ml) in sterile scintillation vials fitted with a thermometer for temperature monitoring. Bacterial suspensions were heated to 40, 50, 60, and 70 °C for 30, 60, or 90 s. These temperatures were selected to simulate temperatures reached during microwave irradiation. To determine culture survival, a 20-μl aliquot of each lysate was plated on chocolate agar plates and incubated overnight at 37 °C.

Lysis of N. gonorrhoeae using microwave irradiation

Fresh dilutions (10^8 CFU/ml) of *N. gonorrhoeae* were lysed in the aforementioned lysing chambers with and without bow-tie lysing structures. The small lysing chambers (Fig. 1B) were used to lyse sample volumes of 500 μl and 1 ml. Sample volumes of 1 ml were also lysed in the large isolators (Fig. 1C) as well as dilutions with a 2-ml sample volume. All samples were exposed to 2.45 GHz microwave irradiation in a 900-W microwave for 30, 60, or 90 s. The bacterial suspensions were exposed to three different microwave powers—10, 30, and 50%—corresponding to 90, 270, and 450 W over the entire microwave cavity. The temperature of the samples was recorded prior to lysing and after each experimental condition (i.e., 10% power for 30 s, etc.). Immediately following microwave irradiation, a 20-μl aliquot of each lysate was plated on selective media and incubated overnight at 37 °C.

Analysis of DNA fragmentation by gel electrophoresis

Prior to gel electrophoresis, the DNA was ethanol precipitated with 0.1× volume of 3 M sodium acetate (pH 5.2) and 2× volume of pre-chilled molecular-grade ethanol, followed by centrifugation. Samples were centrifuged at 14,000 rpm for 20 min, and the supernatant was discarded. DNA pellets were air-dried and rehydrated in 70 μl of DNA rehydration solution (Promega, Madison, WI, USA). To separate DNA from cell debris, all samples were centrifuged at 6000 rpm for 5 min following the pellet rehydration step and prior to gel electrophoresis analysis. To determine DNA fragmentation patterns, 40 μl of each sample was electrophoresed on 1.5% agarose gel in the presence of ethidium bromide.

Analysis of DNA fragmentation using the Agilent Bioanalyzer

To more quantitatively measure the effect of lysing chamber geometry and size on DNA fragmentation, select samples (based on the results from the gel electrophoresis analysis) were also analyzed for DNA fragmentation patterns on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) The Bioanalyzer is an easy-to-use instrument that combines the principles of electrophoresis and flow cytometry to provide sizing, quantitation, and quality control of DNA, RNA, and cells on a single platform. Pre-lysed and lysed samples were prepared for analysis as described in the gel electrophoresis section above, and a 1-μl aliquot of each sample was analyzed on the Bioanalyzer 2100. The results of the analysis reported as the concentration (pg/μl) of each individual double-stranded DNA (dsDNA) fragment present in the sample were used to access the efficiency of the lysing procedure for MAMEF analysis.

Real-time PCR analysis

PCR analysis was carried out to determine how DNA preparation and fragmentation by microwave irradiation affects detection of GC DNA by PCR. Prior to PCR analysis, all samples were centrifuged at 8000 rpm for 10 min to separate DNA from intact bacterial cells. The supernatant was used for reference standard PCR analysis using a previously described 16S real-time PCR assay [22–27]. Briefly, each PCR was performed in a total volume of 50 μl using 30 μl of PCR master mix and 20 μl of sample. PCR master mix contained 25 μl of 2× Taqman universal PCR mix (PE Applied Biosystems, Foster City, CA, USA) and 1.5 μl of 67 μM forward primer (p891: 5′TGGAGCATGTGGTTTAATTCGA3′) and reverse primer (p1033: 5′TGCGGGACTTAACCCAACA3′), and 1 μl of 2.5 U of AmpliTaq Gold (PE Applied Biosystems) and 1 μl of 10 μM probe were added to make up the final master mix before sample was added. Taqman probes for *N. gonorrhoeae* were as follows: 5′VIC-ACAGGTGCTG-CATGGCTGTCGTCAGCT-MGBNFQ3′ and 5′6FAM-TCTCCGGAG-GATTCCGCACATGTCAAAA-MGBNFQ3'. PCR was performed on the ABI 7900 HT sequence detection system (PE Applied Biosystems) with the following cycling conditions: pre-incubation at 50 °C for 2 min, denaturation at 95 °C for 10 min, and 50 repeats at 95 °C for 15 s, annealing/extension temperature at 60 °C for 60 s.

Analysis of vaginal swabs

Detection of DNA target sequences by MAMEF is mediated by the complementary binding of two probes to the target sequences as shown in Fig. 3. The anchor probe contains a sulfhydryl group at its 5′ end, which is used to bind the probe to the metallic nanoparticles. The fluorescent probe contains the fluorophore responsible for the fluorescent signal. In the presence of a complementary DNA target sequence, the three-segment DNA assay is complete, resulting in the fluorescent label being in close proximity to the silver nanoparticles, affording for significantly enhanced fluorescence, that is, optically amplified DNA-based detection.

Vaginal swabs were processed as described previously [3]. Briefly, frozen vaginal swabs were eluted in 2 ml of autoclaved deionized water, and each sample microwave was lysed in a lysing chamber (Fig. 1C) for 35 s at 30% power (270 W). The resulting DNA was separated from cellular debris by a 3-min centrifugation step at 6000 rpm. DNA detection was carried out on silvered wells by hybridization of target GC DNA to complementary probe sequences

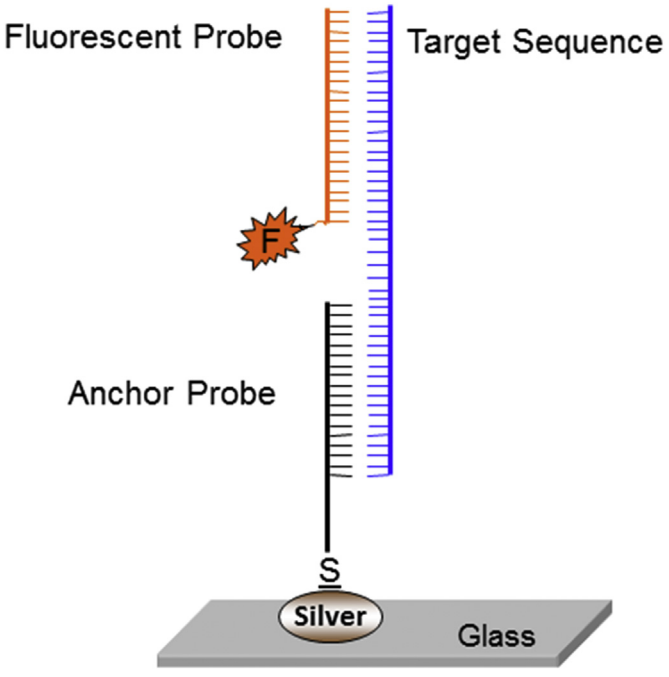

Fig.3. Three-piece MAMEF-based DNA assay.

as shown in Fig. 3. The probe sequences include an anchor probe (5-SH-GCCGTCGTAAGTTAAACAAGG-3) and the fluorescent probe (5-TAMRA-GTCGTTCAGGCGGATATGCGGAC-3).

Results

Determination of bacterial load for DNA fragmentation analysis

To determine the ideal bacterial concentration to evaluate DNA fragmentation patterns, serial dilutions from 10^8 to 10^4 CFU/ml of *N. gonorrhoeae* were lysed by conventional heating at temperatures ranging from 40 to 70 °C and by microwave irradiation for 60 s at 270 W over the entire microwave cavity. Overall, we found that 10^8 CFU/ml was an ideal concentration for DNA fragmentation analysis. DNA fragments were not detected in samples with concentrations less than 10^8 CFU/ml (data not shown).

Effect of osmosis on N. gonorrhoeae lysis and conventional heating on culture survival and DNA fragmentation

Gel electrophoresis analysis of pre-lysed samples suggested that there is a variable degree of cellular lysis prior to conventional heating or microwave-based lysing. To understand how osmotic processes affected cellular lysing, *N. gonorrhoeae* cells suspended in water were incubated for up to 3 h at room temperature. As shown in Fig. 4A, there is a time-dependent effect of cell lysis associated with osmotic processes. However, osmotic processes are not associated with DNA fragmentation. In addition to exploring osmosis-based cellular lysing, the role of conventional heating on culture survival and DNA fragmentation was also investigated. The temperatures used for the conventional heating experiments (40–70 °C) were selected because the temperatures were similar to those reached by organisms in liquid suspension following microwave irradiation (Fig. 5). At 50 °C, a small and variable decrease in culture survival rates was observed, whereas raising the temperature of the cultures to 60 °C (Fig. 5) resulted in complete organism inactivation, suggesting that temperatures higher than 50 °C are required to completely inactivate and kill *N. gonorrhoeae*. Similarly, higher temperatures resulted in increased DNA extraction and partial DNA fragmentation. Extraction of high-molecular weight (MW) genomic DNA increased when the temperature of the cultures reached 60 °C, and complete fragmentation of low-MW DNA fragments (700–1100 bp) was achieved. However, complete fragmentation of high-MW DNA was not achieved even at 70 °C (Fig. 4B).

Effect of microwaves on culture survival

To evaluate the effects of microwaves on gonorrhea organism survival, temperature readings were collected following microwave irradiation and the culture survival results were compared with those by conventional heating at different temperatures (40, 50, 60, and 70 °C). Exposure of *N. gonorrhoeae* cultures to low-power microwaves (90 W) resulted in low temperatures and had no effect on GC culture survival rates (data not shown). However, exposing the cultures to higher microwave power resulted in decreased culture survival rates in a power- and temperature-dependent manner (Fig. 5). When exposed to microwaves the *N. gonorrhoeae* cells were completely inactivated when the temperature of the organisms reached 46 °C, whereas with conventional heating an increased cell survival rate with high variability was observed when the temperature of the cultures reached 50 °C (Fig. 5). Overall, GC culture survival rates were more affected by microwaves than by conventional heating when culture survival rates between the two methods were compared at the same temperature.

Effect of microwaves on DNA isolation and fragmentation

The use of low-power microwaves (90 W) and short exposure times (30 s) had only a minor effect on DNA isolation, for both lysing chambers, when compared with the unlysed sample (Fig. 6A and E). However, the use of longer exposure times (60 and 90 s) and larger lysing chambers (Fig. 1C) resulted in greater DNA preparation when compared with the 30-s exposure time or the unlysed sample (Fig. 6A and E). Furthermore, exposing the cultures to higher microwave power resulted in DNA fragments less than 100 bp in length (Fig. 6C, D, G, and H). At the highest microwave power investigated, complete DNA fragmentation occurred regardless of exposure time (Fig. 6D and H).

Effect of microwave-focusing gold triangles on DNA fragmentation

We investigated whether the use of bow-tie gold triangles deposited on glass slides (Fig. 1) can enhance lysing efficiency by focusing microwaves directly onto the sample volume. At lower microwave power (90 W), very little DNA fragmentation occurs regardless of the use of microwave-focusing triangles (Fig. 6A and E). When GC organisms were microwave irradiated at 270 W in the presence of triangles, complete DNA fragmentation can occur in as little as 30 s (Fig. 6C and G). However, when the same microwave power is applied in the absence of microwave-focusing triangles, no DNA fragmentation was observed (Fig. 6B and F). The

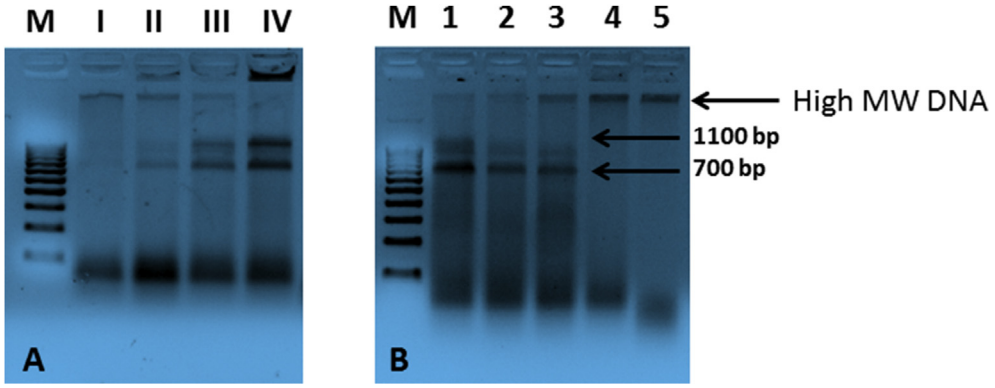

Fig.4. (A) Osmotic effect on *N. gonorrhoeae* cells lysing at room temperature. Lane M: 100-bp DNA ladder; lane I: freshly prepared culture; lane II: 1 h incubation; lane III: 2 h incubation; lane IV: 3 h incubation. (B) DNA fragmentation pattern of *N. gonorrhoeae* by conventional heating. Lane M: 100-bp DNA ladder; lane 1: pre-lyse sample; lane 2: 40 °C; lane 3: 50 °C; lane 4: 60 °C; lane 5: 70 °C. MW, molecular weight.

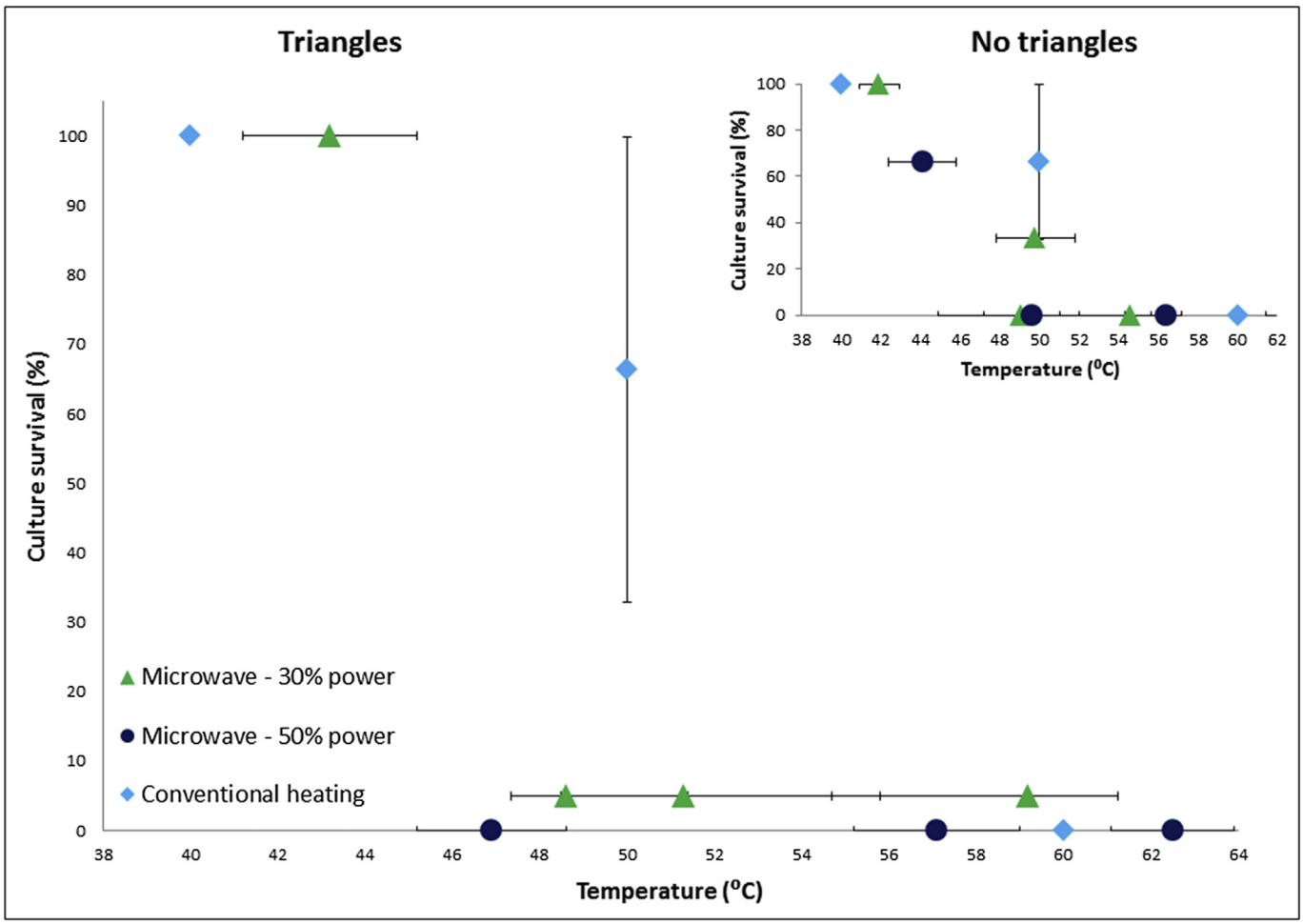

Fig.5. Survival of *N. gonorrhoeae* versus temperature following conventional heating (40, 50, 60, and 70 °C) and microwave irradiation at 10, 30, and 50% microwave power. For simplicity, results at certain temperatures have been omitted because they either did not affect culture survival rates (<38 °C) or completely inactivated bacterial cells (>62 °C).

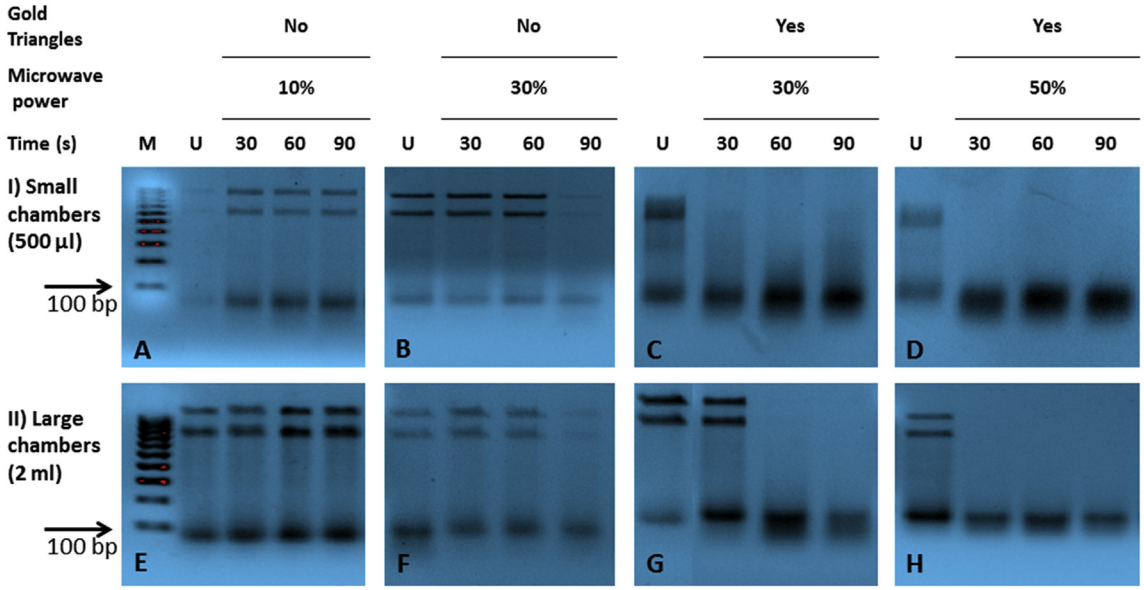

Fig.6. DNA fragmentation pattern of *N. gonorrhoeae* by microwave irradiation: (I) 500-µl lysing volumes using small isolators; (II) 2-ml lysing volumes using large isolators. Lane M: 100-bp DNA marker; lanes U: unlysed sample.

enhancement of DNA fragmentation by microwave-focusing triangles occurs regardless of the type of lysing chamber used (small or large), but it is more striking when small lysing chambers are used (Fig. 6C vs. Fig. 6G).

Effect of lysing chamber geometry on DNA fragmentation

To further elucidate the mechanism of how bow-tie structures help to focus microwaves and enhance lysing efficiency, GC organisms were microwave irradiated in two chambers with different lysing geometries (Fig. 1B and C). Based on our previous studies and thermal imaging [5,20], the prevailing hypothesis is that the bulk of the microwave-driven energy is initially concentrated at the apex of the triangles, resulting in the preferential lysing of cells near that location. When comparing GC organisms microwave irradiated in the presence of lysing triangles (Fig. 6C, D, 6G, and 6H), the concentration of fragmented DNA was greater in cultures that were lysed with the small lysing chambers (Fig. 6C and D) than those lysed in the large lysing chambers (Fig. 6G and H). The observation that small lysing chambers are more efficient for DNA fragmentation than larger lysing chambers is consistent with our previous imaging studies [6,7]. In the small lysing chamber, the entire lysing volume is directly above the microwave-focusing triangles and closer to the apex of the triangles, thereby allowing for a greater number of cells to be near the highest levels of energy. Conversely, when larger isolators were used, the entire sample volume was directly above the triangles but farther away from the apexes of the triangles. The difference in DNA fragmentation efficiency observed when using the small and large lysing chambers is not related to sample volume given that there was no difference in fragmentation efficiency when the larger lysing chambers were used to lyse 1 or 2 ml of sample (data not shown).

Quantitation of dsDNA following microwave irradiation

Based on gel electrophoresis results, we decided to quantitatively analyze the concentration of dsDNA fragments following microwave irradiation in two different lysing chambers. Preliminary results indicated that when the small lysing chambers were used, the highest concentration of dsDNA fragments under 100 bp is achieved after a 30-s microwave irradiation (90 W) exposure. In addition, increasing the power or exposure time resulted in a decrease of the concentration of dsDNA (Fig. 7A). Conversely, higher levels of energy (270 W) were necessary in order to achieve maximum DNA fragmentation with the larger lysing chambers (Fig. 7B). Overall, there appeared to be an inversely proportional relationship between energy level and dsDNA concentration when small isolators were used, which differed from the directly proportional relationship observed with the use of large lysing chambers.

Effect of microwave lysing on PCR

To show that microwave irradiation results in DNA fragmentation, pre- and post-microwave irradiation lysates of *N. gonorrhoeae* were tested by PCR. Exposure of *N. gonorrhoeae* to microwaves for 30 s did not affect the concentration of DNA template available for PCR. However, increasing the exposure time to 90 or 120 s increased the threshold cycle (Ct) of the PCR reactions, suggestive

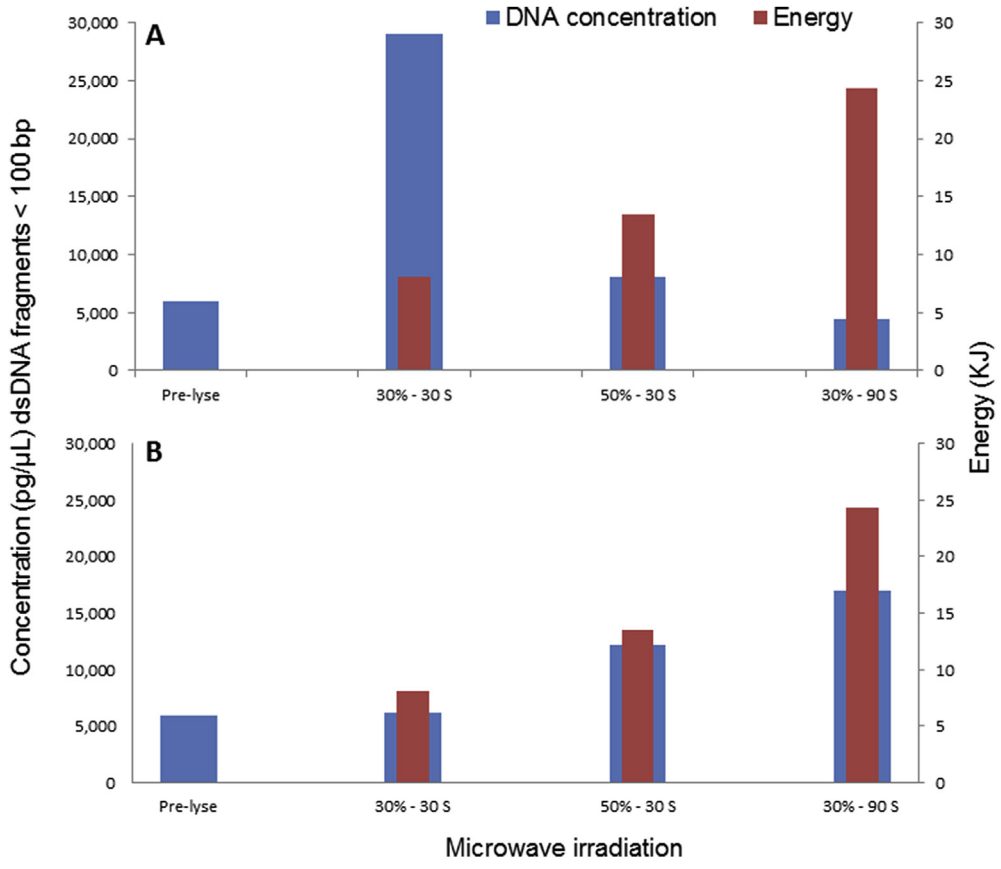

Fig.7. Bioanalyzer-based quantitation of dsDNA concentration (<100 bp) using small (500-µl) lysing chambers (A) and large (2-ml) lysing chambers (B).

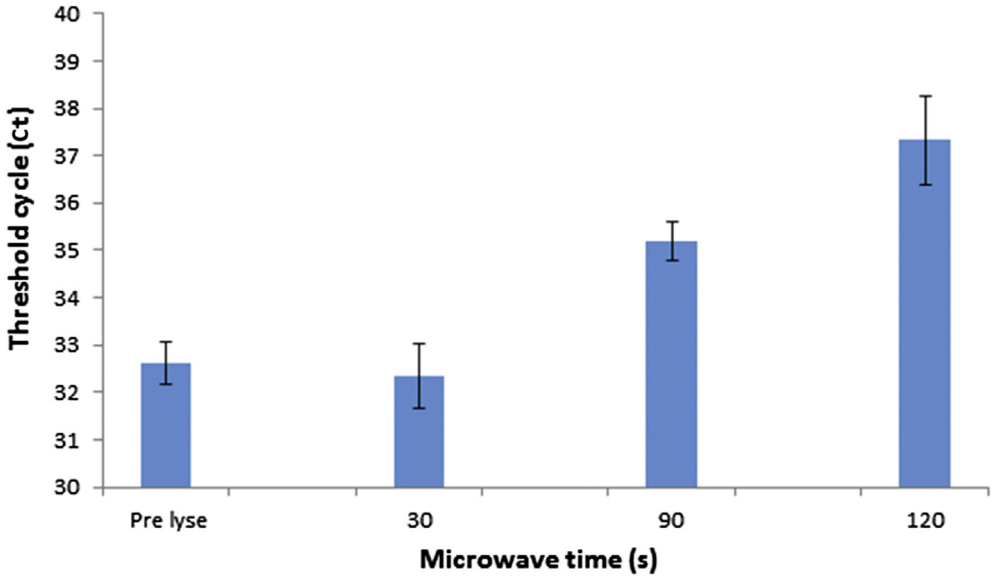

Fig.8. Detection of *N. gonorrhoeae* DNA by PCR before and after microwave irradiation at 30% power.

of a decrease in the concentration of template DNA available for PCR (Fig. 8).

Detection of DNA fragments by MAMEF

To determine whether microwave-based lysing could generate DNA fragments suitable for MAMEF-based analysis, 20 vaginal swabs (6 GC positive and 14 GC negative) were microwave lysed and tested using the previously described MAMEF three-piece DNA assay (Fig. 3). Of the 6 GC-positive swabs tested, MAMEF detected GC DNA in 5 samples.

Discussion

Preparation of DNA for molecular detection and gene expression assays is a time-consuming and often labor-intensive and expensive process. To improve on some of the shortcomings of current DNA extraction methodologies, we have demonstrated the potential utility of a microwave-based system for the rapid extraction and fragmentation of bacterial DNA. There are several notable features about microwave-based lysing, including (i) speed, (ii) lack of specialized instrumentation, (iii) cost, and (iv) application to a variety of molecular methodologies due to its DNA preparation and fragmentation capacity.

Although commercially available kits can be used for the extraction of bacterial DNA, they require a combination of thermal and enzymatic reactions, resulting in long and labor-intensive procedures. Our simple one-step procedure involving a 2.45-GHz household microwave can lyse bacteria in a little as 30 s. As demonstrated by the current study and other studies, the isolated DNA can be successfully used for a variety of molecular approaches, including PCR [15,16], MAMEF [1–6], and next-generation sequencing [17]. Another significant feature of our microwave-based lysing approach is the ability to simultaneously prepare and fragment genomic DNA, noting that this is achieved only by a two-step process using current commercial approaches. The DNA fragmentation patterns obtained from our study are similar to those in previously reported microwave studies [14,17] but without the need of a sophisticated microwave system and yet still carried out in seconds instead of several minutes. This is a significant benefit of our approach. Although we primarily used the extracted DNA for MAMEF analysis, our PCR results suggest that the use of low-power microwaves can result in the isolation of unfragmented DNA, which can invariably be used in conjunction with a variety of other molecular DNA detection strategies such as PCR.

In addition to demonstrating the utility of a microwave-based lysing approach, one of the objectives of this study was to show how bow-tie structures can be used for focusing microwaves and enhancing cell lysis and DNA fragmentation. Culture results suggest not only that microwaves are more effective than conventional heating to lyse bacterial cells but also that the addition of bow-tie structures to the lysing chambers leads to killing and a decrease in organism survival rates. The superior efficacy of small lysing chambers over larger chambers for lysing and DNA fragmentation is likely attributable to the microwave field distribution at the gap of the bow-tie geometries per unit lysing volume. Our previous FDTD-based simulations and thermal imaging studies [6,20] suggested that during microwave irradiation there is a rapid increase in heating rate for solutions in close proximity to the gap of the 12.3-mm disjoined bow-tie structures. In the case of the small lysing chambers, the entire sample is located directly above the bow-tie structures and in closer proximity to the gap of the disjoined lysing triangles than when large lysing chambers are used (Fig. 1B and C).

During our study, we came across several limitations worth noting. First, we were unable to investigate the effect of microwaves on samples with low concentration of bacterial cells ($<10^6$ CFU/ml) due to the sensitivity of the gel electrophoresis technique. However, this should not affect the sensitivity of molecular assays such as PCR and MAMEF because clinical samples (i.e., vaginal swabs) typically have high concentrations of gonorrheal DNA. In addition, in the current study, we tested only a small number of samples by MAMEF following microwave lysing. Our previous studies, however, suggest that sensitivity of MAMEF is high following DNA preparation by microwave irradiation [2–7]. We are currently testing a large cohort of vaginal swabs using our microwave lysing approach and MAMEF technology.

Conclusions

In the current study, we have demonstrated that microwave-based lysing is more effective and faster than conventional heating for the lysing and fragmentation of gonorrheal DNA. Furthermore, the use of disjoined bow-tie structures helps to enhance lysing and DNA fragmentation efficiency by focusing microwaves directly onto the sample. We envision the downstream development of a single protocol that could be used for the lysing of all bacteria regardless of cell wall structure as well as other microbial organisms.

Conflict of interest

The authors can confirm that they have no financial conflicts of interest or otherwise at this time. Three patents are currently filed on the microwave lysing technology. The MAMEF technology has numerous patents both issued and pending in various regions of the world today.

Acknowledgments

This work was supported in whole or in part by National Institutes of Health (NIH)/National Institute of Biomedical Imaging and Bioengineering (NIBIB) 5U54EB007958-09, NIH/National Institute of General Medical Sciences (NIGMS) T32GM066706, and TEDCO MII (Phase 1: #0115-010_2) awarded to C.D.G.

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