Ultrasound in Med. & Biol., Vol. 31, No. 9, pp. 1265–1277, 2005

0301-5629/05/$–see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2005.05.005

● *Original Contribution*

MICROFLUIDIC SONICATOR FOR REAL-TIME DISRUPTION OF EUKARYOTIC CELLS AND BACTERIAL SPORES FOR DNA ANALYSIS

THEODORE COSMO MARENTIS,*† BRENDA KUSLER,‡ GOKSEN G. YARALIOGLU,† SHIJUN LIU,† EDWARD O. HÆGGSTRÖM,†§ and B. T. KHURI-YAKUB†

*Harvard Medical School, Boston, MA, USA; †Edward L. Ginzton Laboratory and ‡Department of Biological Sciences, Stanford University, Stanford, CA, USA; and §Department of Physics, University of Helsinki, Helsinki, Finland

(*Received* 9 *December* 2003, *revised* 5 *May* 2005, *in final form* 24 *May* 2005)

Abstract—Biologic agent screening is a three-step process: lysis of host cell membranes or walls to release their DNA, polymerase chain reaction to amplify the genetic material and screening for distinguishing genetic signatures. Macrofluidic devices commonly use sonication as a lysis method. Here, we present a piezoelectric microfluidic minisonicator and test its performance. Eukaryotic human leukemia HL-60 cells and *Bacillus subtilis* bacterial spores were lysed as they passed through a microfluidic channel at 50 μL/min and 5 μL/min, respectively, in the absence of any chemical denaturants, enzymes or microparticles. We used fluorescence-activated cell sorting and hematocytometry to measure 80% lysis of HL-60 cells after 3 s of sonication. Real-time polymerase chain reaction indicated 50% lysis of *B. subtilis* spores with 30 s of sonication. Advantages of the minisonicator over macrofluidic implementations include a small sample volume (2.5 μL), reduced energy consumption and compatibility with other microfluidic blocks. These features make this device an attractive option for "lab-on-a-chip" and portable applications. (E-mail: theodore_marentis@hms.harvard.edu) © 2005 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound, Sonication, Piezoelectric, Bioeffect, Lysis, Microfluidic, Lab-on-a-chip.**

INTRODUCTION

A variety of fields, such as medical diagnostics or "lab-on-a-chip," can greatly benefit from rapid, on the spot identification of biologic agents. Detection can be accomplished either by DNA hybridization methods (Hianik et al. 2000) that require a large number of DNA copies and, thus, a polymerase chain reaction (PCR) step, or quantitative real-time PCR (RTPCR) (Belgrader et al. 1999). In either case, fast and reliable PCR takes place when cellular hosts are disrupted and their intracellular DNA is made available in solution to interact with the polymerase enzyme. This makes cell lysis a necessary step in the detection process.

A variety of cell lysing methods exist, such as physical, thermal, chemical, enzymatic or mechanical (Kuske et al. 1998; More et al. 1994), which, however, can be too labor-intensive for timely on-site applications (Chandler et al. 2001). Often, they require additional consumables with a certain shelf-life. These consumables may further complicate subsequent PCR and detection steps by altering chemical conditions such as pH, or by inhibiting the necessary molecular interactions (Chandler et al. 2001). To overcome these difficulties, research has focused on sonication or the use of ultrasound (US) to disrupt cellular membranes and spore coats (Belgrader et al. 1999; Chandler et al. 2001; Taylor et al. 2001).

To the authors' knowledge, no comprehensive study is available on threshold pressures required to lyse spores and cells. As reported in the literature, portable sonication implementations developed to date lie in the macrofluidic realm (Belgrader et al. 1999; Chandler et al. 2001; Taylor et al. 2001; Kawai and Iino 2003; Feril et al. 2003; Miller et al. 2003). One disadvantage with these systems is the length of the tubing required to connect the components. Use of microbeads to enhance bacterial spore lysis is also common (Belgrader et al. 1999; Taylor et al. 2001). These beads, however, have to be washed to remove adhered DNA, which complicates the process. Although successful in lysing eukaryotic cells and bacterial spores,

Address correspondence to: Theodore Cosmo Marentis, 107 Avenue Louis Pasteur, Box 239 Vanderbilt, Harvard Medical School, Boston, MA 02115 USA. E-mail: theodore_marentis@hms.harvard.edu

size and energy consumption preclude macrofluidic implementations from delivering a truly portable screening device (NASA Fundamental Space Biology homepage, available at: http://fundamentalbiology.arc.nasa.gov/EP/EPpres2.html).

Unfortunately, *Bacillus anthracis* (anthrax) has recently re-emerged as a potential threat in the hands of the wrong people. Its spores are easily cultivated *en masse*, stored for decades in their vegetative state and can be easily aerosolized (Inglesby et al. 1999). This has strengthened efforts to improve the speed and specificity of available biologic agent detection methods, as well as the portability of the testing equipment (DARPA, Defense Advanced Research Project Agency homepage, available at: http://www.darpa.mil/MTO/bioflips). To ensure specificity, detection methods screen for genetic signatures that confer toxicity to anthrax (Read et al. 2003; Ivanova et al. 2003), which leads back to the problem of cell lysis.

Meanwhile, the concept of "lab-on-a-chip" is evolving (Knight 2002; Anderson and van den Berg 2003). A variety of new microelectromechanical and microfluidics implementations are being developed, such as electro-osmotic pumps (Zeng et al. 2001), ultrasonic mixers (Yaralioglu et al. 2004), small PCR chambers (Kopp et al. 1998; Lagally et al. 2001; Sun et al. 2002) and electrical readout DNA arrays (Umek et al. 2001; Patolsky et al. 2001; Thewes et al. 2002). These microfluidic blocks can be patterned on silicon and cascaded next to each other. This allows complex biologic and chemical experiments to be performed on a computer-controlled chip, with minute sample volumes, thus reducing reagent and labor costs. Currently, no efficient on-a-chip lysis block exists. Lysing has so far been performed macrofluidically, comprising a bottleneck for any "lab-on-a-chip" assay (Chandler et al. 2001).

We present a microelectromechanical-based piezoelectric microfluidic minisonicator operated in the 380 MHz range to fill this need for a miniaturized lysing device. The device lyses cells in the absence of chemical, biologic or microparticle agents in a continuous manner as they flow through a 50-μm channel. This allows for real-time continuous monitoring applications. We tested the minisonicator with eukaryotic HL-60 cells that lack a cell wall and then proceeded to vegetative *B. subtilis* bacterial spores, which are considered to be difficult to lyse (Taylor et al. 2001). To our knowledge, this is the first microfluidic device that disrupts cells with US.

MATERIALS AND METHODS

Device design and fabrication

We have developed a microfluidic channel with integrated transducers (Jagannathan et al. 2001, 2003a, 2003b). The geometry of the device is shown in Fig. 1. The device is composed of two parts, the channel and the transducers. The channel was fabricated on a glass substrate, whereas the transducers were fabricated on a quartz substrate. The substrates are 20 $\times$ 25 mm in size. Glass was chosen for the channel material because it is transparent and can be micromachined. The channel was formed by wet-etching the glass substrate in a 50:1 hydrofluoric acid solution (Gallade Chemical Inc., Escondido, CA, USA). A 0.5-μm layer of photolithographically patterned polysilicon was used as a masking layer. The dimensions of the channel were 10 mm in length, 500 μm in width and 500 μm in depth. Hence, the cross-section was 0.25 mm^2, and the volume was 2.5 mm^3, giving a flow-velocity of 4 mm/s at 1 μL/s flow. These dimensions should be compared with those of 10 μm by radius HL-60 cells (van Dujin et al. 1998) and 0.7 μm by diameter and 2 to 6 μm by length *B. subtilis* spores (Janosi et al. 1998).

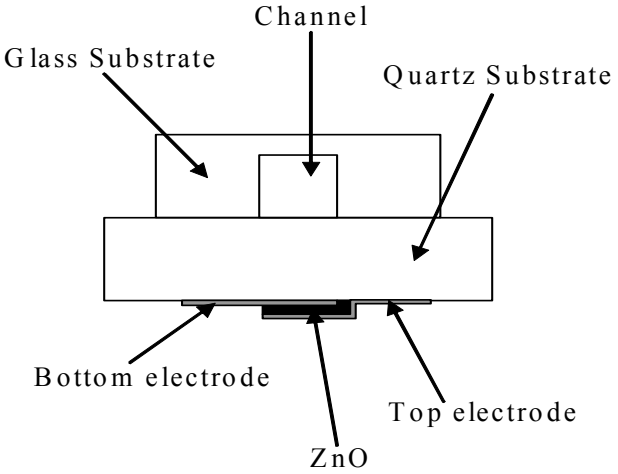

Fig. 1. Cross-section of the microfluidic channel.

The piezoelectric transducers used in our experiments were integrated onto the channel floor by depositing a layer of zinc oxide between two layers of gold on a quartz substrate. Quartz was chosen as a substrate material because of its low loss coefficient for acoustic waves. Figure 2 describes the fabrication steps of the transducer. First, a 0.1-μm film of gold was sputtered and patterned over a glass substrate to serve as one of the electrodes of the transducer (steps 1, 2). This step was followed by deposition of 8 μm of zinc oxide by means of a magnetron sputtering technique (step 3) (Khuri-Yakub et al. 1981) using a shadow mask. Finally, another 0.1-μm thick layer of gold was deposited and patterned using lift-off to serve as the top electrode of the transducer (steps 2, 4, 5, 6). The size of each of the nine piezoelectric transducers was 500 μm by 500 μm, leading to a 45% coverage of the channel floor. The trans-