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A facile one-step method for cell lysis and DNA extraction of waterborne pathogens using a microchip

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ABSTRACT

Globally, waterborne organisms are the primary causative agents for the transmission of various forms of diarrheal diseases. For accurate diagnosis, molecular tools have gained considerable attention in the recent past. Molecular tools require DNA as the starting material for diagnosis, and hence, a prerequisite is the quality and integrity of DNA. To obtain high quality DNA rapidly, we have fabricated a microchip in poly(dimethyl siloxane) (PDMS) by soft lithography process. The microchip facilitated in-flow coating of chitosan on the magnetic nanoparticles, which under external mechanical vibration caused cell lysis and released DNA in the supernatant. The released DNA was captured by the nanoparticles owing to its positive charge (chitosan coating). The magnetic nanoparticle-DNA complex was then isolated from the in-flow matrix using permanent magnet, Further, removal of the cell debris, proteins, and carbohydrates was done using wash buffer. DNA extracted using the microchip was pure with absorbance (260/280) ratio of 1.77 ± 0.04, as compared to 1.79 ± 0.03 obtained by TRIzol method. The complete isolation of the DNA using the microchip took ~ 15 min as against > 2 h with a TRIzol method. Six gram-negative waterborne pathogens were used to demonstrate the efficacy of the microchip based DNA extraction process. The integrity of the isolated DNA was assessed by amplifying the 16S rRNA gene using Com1 and Com2 universal primers. The presence of a band at 407 bp on gel electrophoresis confirmed the amplified product. Further, the gel image was used for quantification of the amplified product using ImageJ software. Higher regression values obtained using microchip confirmed better quality and integrity of the extracted DNA as opposed to the conventional method. The lower (< 2%) relative standard deviation values obtained from the data suggested that the microchip process was reproducible. The quality and integrity of the obtained DNA proved the simplicity, rapidity, and sensitivity of the microchip-assisted DNA extraction process.

1. Introduction

Molecular diagnostics have gained considerable attention in the recent past especially for the detection of waterborne pathogens (Ho et al., 2012; Mairhofer et al., 2009). Waterborne organisms like *Escherichia coli*, *Salmonella typhimurium* (non-typhoidal), *Shigella boydii*, *Pseudomonas aeruginosa,* etc. are the primary causative agent in the transmission of the disease globally. For molecular diagnosis of these organisms, obtaining good quality DNA is essential. Traditionally, process of acquiring pure DNA is cumbersome along with the requirement of corrosive chemicals, creating ample opportunity for degradation (Legendre et al., 2008). Isolation of DNA primarily requires lysis of the cells which are traditionally done using enzymes (lysozyme), chemical lytic agents (detergents), and mechanical forces (bead milling, sonication, etc.). Hence, a facile, rapid and sensitive method for the extraction of high purity DNA for molecular diagnostics is the need of the hour.

Microfluidic platform offers several advantages as opposed to the traditional approach for DNA isolation viz. rapid isolation, low reagent consumption, and involvement of fewer steps. On-chip cell lysis has been demonstrated by mechanical methods using nanostructure barbs (Di Carlo et al., 2003), electrical (Gao et al., 2004) and thermal energies. Anthrax spores were lysed using a mini-sonicator system and the DNA was extracted (Belgrader et al., 1999). Sonication imparts non-uniform and uncontrolled mechanical force for disruption of cells which can lead to degradation of DNA. The chemical method of lysis employs harsh chemicals to disrupt cell walls in addition to chaotropic reagents to denature proteins (Tan and Yiap, 2009). Interference of these chemical reagents may result in a reduced quality of DNA. Moreover, the process is labor-intensive and requires expensive con-

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sumables. The electrical method requires a high electric power (up to 2 kV/cm) to disrupt the cells, which becomes a serious impediment (Gao et al., 2004; Han et al., 2003). Imparting heat to disrupt cell walls can be a suitable alternative, however, uncontrolled heat can result in the interaction of denatured proteins with released DNA, degrading the quality (Abolmaaty et al., 1998).

Researchers have also attempted to extract and isolate DNA using solid phase extraction (SPE) principle (Mahalanabis et al., 2009). DNA captured from bacterial cells was successfully demonstrated using micro-pillars coated with silica gel (Cady et al., 2003), continuous phase-transfer magnetophoresis (Karle et al., 2010), Laser-Irradiated Magnetic Bead System (LIMBS) (Lee et al., 2006) etc. DNA from sperm cells has also been extracted using microchip using packed silica channel (Bienvenue et al., 2006). Similar to SPE, liquid phase extraction has also been demonstrated to isolate DNA from a low cell number (Zhang et al., 2013). The reported techniques are limited in terms of time required for extraction, pre-processing steps, recovery of the extracted DNA, contaminants during elution, use sophisticated instrumentation and processing.

Nanoparticle-based DNA extraction is employed to overcome the deficiencies of time, reagent consumption, and purity of obtained DNA. Nanoparticles are best suited for the isolation of DNA owning to the high surface to volume ratio, sedimentation free movement, and modifiable surface charge. Cheong et al. (2008) have demonstrated gold nanoparticle-mediated DNA capture (starting with pre-lysed cells) in one-step to improve capture efficiency and the quality of DNA. However, a major hurdle prevails to rapidly extract high-quality DNA without any pre-or post-processing of the samples.

We propose a method for microchip based cell lysis and DNA extraction from waterborne pathogens. In our approach, we have employed chitosan-coated magnetic nanoparticles (Lanthanum Strontium Manganese Oxide, LSMO) (Kulkarni et al., 2015) for effective bacterial lysis under mechanical vibrations. After cell lysis, the released DNA was captured on the positively modified magnetic nanoparticles and purified using a permanent magnet. An advantage of using magnetic nanoparticles is the ease of isolation of DNA from cellular components. Moreover, nanoparticles act as the projectile during mechanical vibrations triggering controlled cell lysis. DNA isolated using the microchip was assessed for its recovery, purity, and integrity in comparison to the TRIzol (conventional) method.

2. Material and methods

2.1. Fabrication of the microchip

The microchip for cell lysis and DNA extraction was fabricated using PDMS by template assisted soft lithography technique (Agrawal et al., 2012; Kamat et al., 2015). The fabricated microchip has two inlet channels (ϕ500 μm), which permit the reactants to flow into the reaction chamber (ϕ8 mm) containing a microneedle (Fig. 1a, b). The microneedle was fabricated by mixing iron oxide powder in PDMS, followed by curing and cutting in rhombic shape of specific dimension (detailed protocol described in our previous publication, (Kamat et al., 2015)). A third inlet (ϕ500 μm) is placed approximately in the middle of the U-shaped (50 mm) channel. A mechanical vibrator (ϕ12 mm; 3 VDC coin vibration motor model no. 308-100) is placed close to the U-shaped channel (Fig. 1) to impart vibrations to the liquid suspension. A permanent disk magnet (ϕ10 mm; Lithium Niobate with B: 0.2 T) was embedded beneath the magnetic capture chamber (ϕ8 mm) connected to the outlet channel (ϕ500 μm).

2.2. Synthesis of chitosan coated LSMO nanoparticles and its characterization

A stock solution containing 10 mg/mL LSMO nanoparticles (magnetic) in deionized water (average size ~ 50 ± 10 nm, zeta potential −13 mV) (Ravi et al., 2007) was admixed with 0.5 mg/mL chitosan (Sigma-Aldrich, USA, deacetylation: 75–85%; viscosity: > 400 mPa s) dissolved in 1% acetic acid (pH 3.3) and was kept overnight. A stock solution of 1.6 mg/mL sodium tripolyphosphate (TPP, Merck) in deionized water was used as a cross linker. 1 mL of the stock solution containing chitosan and LSMO nanoparticles was introduced from inlet-1 and TPP from inlet-2 in the ratio of 2.5:1 (Kamat et al., 2015) using a syringe pump (11 Pico Plus Elite programmable syringe pump, Harvard Apparatus, USA). A constant flow rate and rotational acceleration of 100 μL/min and 1000 rpm respectively were maintained throughout the reaction. Size and Zeta potential of the chitosan-coated LSMO (C-LSMO) nanoparticles were measured using Nanosight (LM10, Malvern, UK) and Delsa Nano (Beckman Coulter, USA) respectively. Scanning electron microscopy (SEM) was carried out using Zeiss EVO MA-15 instrument with a LaB_6 filament as the source. Transmission electron microscopy (TEM) was performed using FEI Technai-30 system, Oregon, USA operated at 300 kV. Nature of chemical bonding and binding strength of chitosan with LSMO was evaluated using Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Spectrum One Spectrometer) and Thermogravimetric analysis (PerkinElmer TGA 4000) respectively.

2.3. Determining DNA capture efficiency of C-LSMO nanoparticles

The capture efficiency of C-LSMO nanoparticles was evaluated by spiking 5 μg pure genomic DNA isolated from 10^8 *Escherichia coli* NCIM 2931 (acquired from National Chemical Laboratory, Pune, India). Varying concentration of LSMO (0.25–3 mg/mL) nanoparticles and chitosan (0.025–0.3 mg/mL) was mixed (1:10 w/w chitosan: LSMO) and injected through inlet. The C-LSMO nanocomplex was allowed to interact with 5 μg DNA diluted in 100 μL nuclease-free water injected from the inlet-3 (Fig. 1) at a flow rate of 100 μL/min.

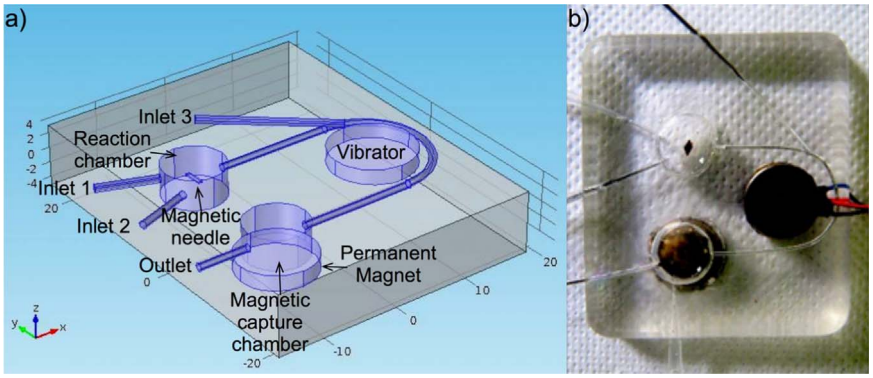

Fig. 1. a) Schematic of the fabricated microchip depicting inlets, reaction chamber with micro needle, vibrator, Magnetic capture chamber and outlet. b) Microchip fabricated in PDMS using soft lithography.