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An electro-conductive plane heating element for rapid thermal lysis of bacterial cells

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ABSTRACT

In the process of developing a point-of-care testing device, we fabricated a plane heating element using carbon paste capable of thermal lysis. The plane heating element can generate heat of 150 °C within 60 s at 0.4 A power supply within 8 W. The developed plane heating element was very effective in the extraction of DNA from both Gram-negative and Gram-positive bacteria which was further confirmed by agarose gel electrophoresis. In this approach, we aimed to eliminate expensive, time-consuming and labor-intensive procedures for the lysis of bacterial cells to extract nucleic acids to detect pathogenic bacteria accurately and swiftly.

1. Introduction

Globally, bacterial infections pose a major threat of morbidity and mortality with millions of deaths and hospitalizations each year. Therefore, early detection of pathogenic bacteria responsible for the manifestation of the disease is essential to prevent difficulties that arise out of the progression of the disease and is advantageous for formulating an effective therapy (Yan et al., 2017). Detection of bacterial pathogens with extreme sensitivity allows rapid diagnosis of disease and recommendations for treatment, but these techniques are not compatible with low resource settings, or the point-of-care testing (POCT) (Drancourt et al., 2016; Heiniger et al., 2016; Kim et al., 2018). This difference in technology availability affects not only health outcomes, but increases the burden of infectious diseases in developing countries (Wang et al., 2017; Zhang et al., 2017). In an effort to address these challenges, paper-based microfluidics has been studied because of the small sample, ease of manufacture, fast assay times and low-power consumption (Akyazi et al., 2017; Gao et al., 2004; Kim et al., 2009).

Sample preparation, which involves bacterial cell lysis to extract nucleic acids for downstream detection processes a critical first step to achieving successfully POCT design. Many physical and chemical methods have been established to prepare samples, but most of the methods require sophisticated equipment, chemical reagents and multiple purification steps (Ho et al., 2006; Nan et al., 2014). In sonication as a physical method, ultrasonic waves become weak with increase in distance from sonicator, so sonication method does not give consistent lysis result and it is difficult to integrate with the next process (Shehadul Islam et al., 2017). In chemical methods as a non-mechanical method, chemicals such as acids, bases, detergents and solvents. The chemical lysis breaks down the cell membrane effectively, but this method requires a subsequent removal process because residual chemicals prohibit the following process (Balasundaram et al., 2009). The standard phenol-chloroform method for bacterial DNA extraction is very time-consuming and labor-intensive. To overcome disadvantages of these chemical approaches, thermal lysis approach is one, which does not require reagents, further purification steps, and is easy to integrate with subsequent detection processes (Afghani and Stutman, 1996; Baek et al., 2010; Holmes and Quigley, 1981). In this study, in the process to develop devices for POCT of bacterial pathogens, we present rapid heat-only bacterial lysis (30 s) with an easy fabrication method of plane heating element that can be used to extract DNA. The major advantage of the lysis method is that it does not require chemicals and laborious purification steps.

2. Materials and methods

2.1. Plane heating element fabrication

In this study, a carbon paste which contained 8% of carbon components was used to lyse the bacterial cells. The carbon paste was kindly provided by Prof. TaeYoung Kim of Gachon University, South Korea. To make the plane heating element, we took a glass fiber filter paper (Glass Fiber Filters, Advantec, GC-50, 150 mm) as a substrate because of its uniformity, binder-free, filtration property and chemical resistance (Vogelstein and Gillespie, 1979). We fixed the glass fiber filter paper on 160 mm × 160 mm stainless plate with 3 M scotch tape.

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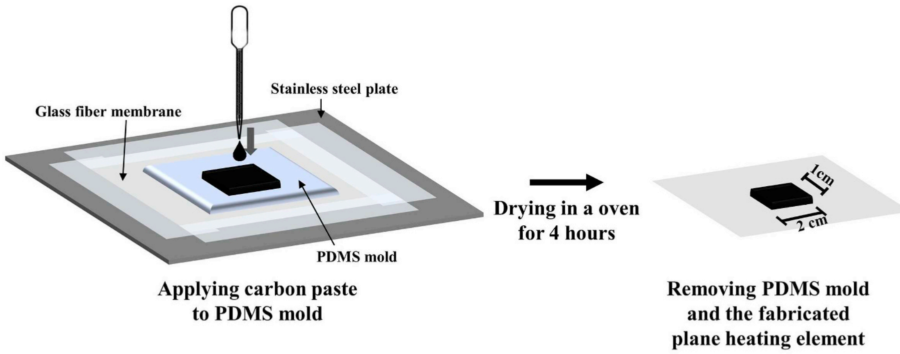

Fig. 1. The fabrication of plane heating elements.

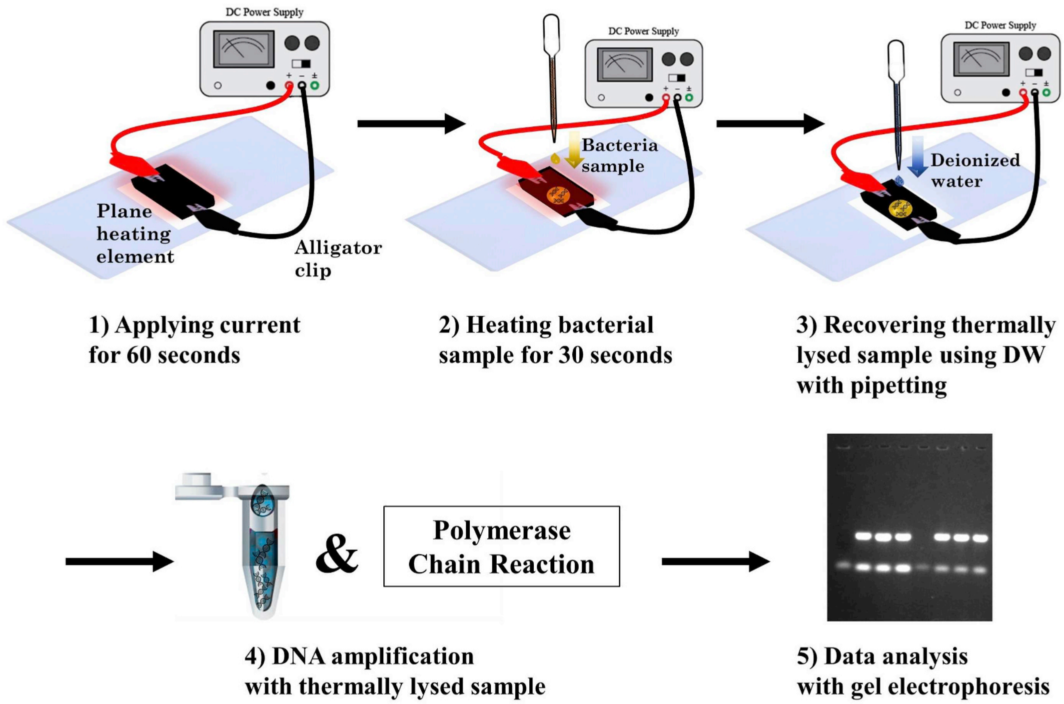

Fig. 2. The whole process of thermal lysis with the plane heating element.

Table 1
Thickness of the plane heating elements.

Thickness of PDMS mold		1 mm	1.5 mm	2 mm
Thickness of each plane heating elements	1	0.292	0.3	0.392
	2	0.297	0.305	0.385
	3	0.283	0.302	0.380
	4	0.293	0.301	0.393
	5	0.292	0.296	0.379
	6	0.291	0.306	0.370
	7	0.291	0.299	0.379
	8	0.280	0.293	0.380
	9	0.286	0.308	0.378
	10	0.289	0.310	0.390
	Average (mm)	0.289 ± 0.005	0.302 ± 0.005	0.383 ± 0.007

Table 2
Resistance of the plane heating elements.

Thickness of PDMS mold		1 mm	1.5 mm	2 mm
Resistance of each plane heating elements	1	62.02	43.31	33.54
	2	58.51	39.24	32.30
	3	63.35	39.27	14.78
	4	63.43	39.91	15.28
	5	60.76	38.80	10.16
	6	61.21	36.49	10.83
	7	62.62	42.43	33.58
	8	68.01	46.59	32.38
	9	65.26	37.39	22.66
	10	65.00	35.01	31.59
	Average(Ω)	63.017 ± 2.66	39.844 ± 3.44	23.71 ± 10.03

Polydimethylsiloxane (PDMS) molds with 1, 1.5 and 2 mm thickness and 20 × 10 mm area were placed on a glass fiber filter paper, and then the PDMS mold was filled with carbon paste and fully dried in an oven at 100 °C for 4 h (Fig. 1). The thickness of the fabricated plane heating elements was measured with a micrometer and resistance with a resistivity meter (MCP - T 700, Co. Loresta - GX).

2.2. Heating properties measurement of the plane heating element

The temperature of the plane heating element was measured using a thermal imager (Ti 32, Fluke Corporation, USA) with a range from −20 °C to 203 °C at the center of the plane heating element. The plane heating element was connected to a DC power supply (6303D, Topward) using an alligator clip. To measure the temperature, we used

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