Food Control 57 (2015) 238–245

Contents lists available at ScienceDirect

Food Control

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

Miniaturized polymerase chain reaction device for rapid identification of genetically modified organisms

Minh Luan Ha [a], Nae Yoon Lee [a, b, *]

[a] Department of BioNano Technology, Gachon University, 1342 Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do 461-701, Republic of Korea
[b] Gachon Medical Research Institute, Gil Medical Center, Inchon 405-760, Republic of Korea

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form
6 April 2015
Accepted 8 April 2015
Available online 1 May 2015

Keywords:
Genetically modified organism (GMO)
Soybean
Polycarbonate (PC) microdevice
Surface hydrophilic/hydrophobic modification
Flow-through polymerase chain reaction (PCR)
35S promoter
bar gene

ABSTRACT

In this study, a polycarbonate (PC) microdevice functioning as a reactor for the polymerase chain reaction (PCR) was fabricated for rapid identification of genetically modified organisms (GMOs). The PC microdevice was fabricated by first modifying its surface with an amine-functionalized alkoxysilane, namely bis[3-(trimethoxysilyl)propyl]amine (bis-TPA), to obtain a hydrophilic surface. Coating of bis-TPA on PC was realized by forming a urethane linkage between the amine terminals of the bis-TPA with the carbonate backbone of PC by aminolysis. This surface enabled the thermal bonding of two PC substrates at a relatively low temperature and atmospheric pressure, thereby maintaining the structure of the microchannel in high resolution. Next, the surface of the microchannel was further treated with a fluorosilane, namely tridecafluoro-(1,1,2,2-tetrahydrooctyl)-triethoxysilane (FTES), to obtain a hydrophobic surface inside the microchannel. This modification was realized by the hydrolysis and subsequent condensation of the alkoxy terminals of both bis-TPA and FTES to form a robust siloxane (Si—O—Si) bond. The hydrophobic microchannel improved the PCR performance by stabilizing the fluid flow, particularly under heated conditions, when the flow-through PCR was conducted on a microdevice. Using the microdevice, the 35S promoter sequences and *bar* gene, which are commonly used for the identification of GMOs, were successfully amplified, resulting in the detection of 234 and 504 bp gene fragments for the 35S promoter sequences and 261 bp gene fragment for the *bar* gene from the genomic DNA extracted from the leaves of GM soybean. This process took approximately 30–35 min, which was approximately 3-fold faster than when using a conventional thermal cycler.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Genetically modified organisms (GMOs) are defined as organisms whose genetic materials have been altered by genetic engineering. The emergence of GMO products has brought numerous benefits such as the enhancement of crop productivity and the increase in nutritional content (Gachet, Martin, Vigneau, & Meyer, 1999). However, besides the above-mentioned advantages, GMOs also pose potential risks to human health (Dona & Arvanitoyannis, 2009). Currently, several methods are available for the identification of GMOs, such as protein-based and nucleotide-based amplification techniques, among which are enzyme-linked immunosorbent assay (ELISA), lateral flow strip, polymerase chain reaction (PCR), western blot analysis, as well as the detection of specific promoter and terminator sequences (Brett, Chambers, Huang, & Morgan, 1999; Dong et al., 2008; Liu et al., 2004; Miraglia et al., 2004; Permingeat, Reggiardo, & Vallejos, 2002; Rudi, Rud, & Holck, 2003; Ujhelyi et al., 2008; Xiao et al., 2012; Zhang & Guo, 2011). Among the aforementioned methods, nucleotide-based amplification by polymerase chain reaction (PCR) is the most commonly adopted method worldwide (Meyer, 1999; Pauli et al., 2001). Although PCR is one of the most time-consuming and labor-intensive processes for genetic analyses, however, this method is indispensable for enhancing the sensitivity of target detection. It has been reported that a large number of GMOs share the same promoter of the 35S subunit of ribosomal RNA of cauliflower mosaic virus (P35S) (Lin, Chiang, & Shih, 2001; Liu, Xing, Shen, & Zhu, 2005), which can be used as a common marker for the detection of GMOs. Besides the 35S promoter sequences, *bar* gene, which is originally derived from the soil bacterium *Streptomyces hygroscopicus* and used to engineer herbicide-resistant plants, was used to identify GMOs.

* Corresponding author. Tel.: +82 31 750 8556; fax: +82 31 750 8774.
E-mail addresses: hmluan87@gmail.com (M.L. Ha), nylee@gachon.ac.kr (N.Y. Lee).

http://dx.doi.org/10.1016/j.foodcont.2015.04.014
0956-7135/© 2015 Elsevier Ltd. All rights reserved.

With the development of microfluidic technology, many researchers have endeavored to miniaturize the PCR process (Chow, 2002; Felton, 2003; Stone & Kim, 2001). Among the many biological reactions with miniaturization potential, flow-through PCR on a miniaturized platform significantly facilitated the speed of DNA amplification owing to the high surface-to-volume ratio, smaller thermal capacity, and larger heat transfer rate realized inside the microchannel. Moreover, this technology can take advantage of a reduced use of sample and reagents, potential for parallel reactions, and enhanced portability. For this reason, many researchers have attempted to develop microfluidic PCR devices (Belgrader et al., 2003; Burns et al., 1996; Chen, Wang, Young, Chang, & Chen, 1999; Lagally, Emrich, & Mathies, 2001; Lagally, Medintz, & Mathies, 2001; Matsubara et al., 2005; Nakano et al., 1994; Yang et al., 2002), which have been applied in the fields of biology, chemistry, medicine, forensic science, food technology, and environmental science. Polycarbonate (PC) has been widely utilized as the material of choice for fabricating microdevices because of its high impact resistance, low moisture absorption, relatively high glass transition temperature (T_g), and optical transparency (Chen et al., 2005; Liu et al., 2001; Yang et al., 2002; Zhang, Xu, Ma, & Zheng, 2006). Among these advantages, the high T_g (~145 °C) of PC, in particular, makes it suitable for withstanding the reaction temperatures as high as 95 °C that are required for the denaturation of nucleic acids.

In this study, we fabricated a PC microdevice to perform flow-through PCR for rapid identification of GM soybeans. To facilitate the thermal bonding of two PC substrates without deformation or collapse of the microchannel, the PC surface was first hydrophilically modified. The hydrophilic modification of PC was easily realized by forming a urethane linkage employing amine-terminated chemicals because the amine functionality can react with the carbonate backbone of PC by aminolysis (Wu & Lee, 2014; Zhang, Trinh, Yoo, & Lee, 2014). Oxidation of the alkoxy terminals of the amine-terminated chemicals on both PC surfaces ensures bonding through the formation of robust siloxane bonds (Si—O—Si) under relatively mild conditions such as atmospheric pressure and a temperature lower than the T_g of PC. After assembling two PC substrates, the hydrophilic PC surface inside the microchannel was further modified with a hydrophobic fluorosilane (Wildes et al., 1999). Hydrolysis and condensation between the alkoxy terminals of the fluorosilane and the hydrophilically modified PC enabled the hydrophobic modification of the microchannel. This hydrophobic coating of PC ensured stable fluid flow inside the microchannel under heated conditions (Jankowski, Ogonczyk, Kosinski, Lisowski, & Garstecki, 2011). This device was adopted to perform flow-through PCR on a microdevice for the detection of GM soybeans, paving the way for the development of a portable platform for the rapid identification of GM products.

2. Material and methods

2.1. Materials

PCR reagents such as *Taq* polymerase, PCR buffer solution, and dNTPs were purchased from Promega. Bovine serum albumin (BSA; V fraction), bis[3-(trimethoxysilyl)propyl]amine (bis-TPA), and (tridecafluoro-1,1,2,2-tetrahydrooctyl)-triethoxysilane (FTES) were purchased from Sigma. TAE buffer (50×) was purchased from Biosesang. A 100 bp DNA size marker was purchased from Takara and agarose powder was purchased from BioShop. Ethidium bromide (EtBr) (Loading STAR) was purchased from DyneBio. Poly(-dimethylsiloxane) (PDMS) (Sylgard 184) and prepolymer were purchased from Dow Corning. An *AccuPrep* GMO DNA extraction kit was purchased from Bioneer for the extraction of genomic DNA from plants. A PC substrate with a thickness of 2 mm was purchased from Goodfellow. Ethanol (94%) and isopropyl alcohol (IPA) (99.5%) were purchased from Daejung Chemical & Metals. GM soybeans were kindly donated by the National Academy of Agricultural Science, Korea.

2.2. Microdevice design and fabrication

Fig. 1A shows the concept for performing a rapid identification of GM soybeans using the PC microdevice having serpentine microchannel engraved using a computer numerical control (CNC) milling machine. The width, depth, and total length of the microchannel were designed to be 300 μm, 100 μm, and 210 cm, respectively, comprising 30 thermal cycles. The overall footprint of the microdevice was 50 × 40 mm. In this experiment, two-temperature PCR was performed by conducting annealing and extension at the same temperature (Nakayama et al., 2006; Sun, Yamaguchi, Ishida, Matsuo, & Misawa, 2002). The inlet and outlet ports (d = 2 mm) were punched using a drilling machine. Silicone tubes (i.d. 1 mm, o.d. 2 mm) were inserted into the ports and were adhered to the substrate using PDMS glue.

2.3. Bonding strategy

Fig. 1B–D shows the procedures for the hydrophilic and subsequent hydrophobic modifications of the PC surface and PC—PC bonding following hydrophilic modification. In brief, the PC substrate was cleaned by sonication in water for 10 min, dipped into the IPA solution and shaken for 5 s at room temperature, and dried. Then, the PC surface was allowed to react with a 5% (v/v) bis-TPA solution in ethanol at room temperature for 1 min (Fig. 1B). Two PC substrates were embossed at 130 °C under 0.1 MPa for 30 min (Fig. 1C). In this way, the PC—PC assembly was realized under relatively mild conditions, i.e., using a lower temperature and pressure than typically applied in thermal bonding (Jang, Park, & Lee, 2014; Ogończyk, Wegrzyn, Jankowski, Dabrowski, & Garstecki, 2010). After assembly, the microchannel was further treated with an ethanolic solution of FTES at room temperature for 1 h (Fig. 1D) (Jang, Park, & Lee, 2014). To examine the optimum condition for hydrophobic modification of the surface, the concentration of FTES were varied (1%, 3%, 5%, 10%, and 15%) (Jia, Fang, & Fang, 2004; Matinlinna, Areva, Lassila, & Vallittu, 2004).

2.4. Surface characterization

2.4.1. Contact angle measurement

The water contact angles were measured by the sessile drop technique using a Phoenix 300 contact angle measuring system (Surface Electro Optics, Korea). The results were analyzed using the ImagePro 300 software. The measurements were performed five times and averaged.

2.4.2. FTIR analysis

FTIR analyses were conducted using an FT/IR 4100 spectrometer (JASCO, Japan). Transmission data were acquired in the range of 4000–400 cm^{-1} (100 scans at a 2 cm^{-1} resolution).

2.5. Bond strength analysis

The pull strength of the bonded PC assemblies (2 × 2 cm) was measured using a texture analyzer (QTS 25, Brookfield, Middleboro, MA). After coating both substrates with 5% bis-TPA, two PC substrates were partially assembled with an overlap length of 2 mm or an overlap area of 40 mm^2. Bonding was performed at 130 °C under 0.1 MPa for 30 min. Holes were punched on both PC substrates

Download English Version:

https://daneshyari.com/en/article/6390725

Download Persian Version:

https://daneshyari.com/article/6390725

Daneshyari.com