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Label-free detection of bacterial RNA using polydiacetylene-based biochip

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ABSTRACT

We developed a simple and effective polydiacetylene-based, label-free multiplex DNA chip for the detection of various pathogenic microorganisms. A novel immobilization method of PDA vesicles on glass slides was exploited using α-cyclodextrin (α-CD). The surface topography of the efficiently immobilized PDA vesicles was confirmed using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Then, oligonucleotides complementary to rRNAs of three pathogenic bacteria were conjugated to the PDA vesicles. Finally, crude lysate of pathogenic bacteria was applied to the PDA biochip. The pathogenic bacteria were specifically detected by DNA–RNA hybridization in an hour. The new PDA sensor was effective in detecting multiple pathogenic bacteria easily and accurately without rigorous purification, amplification, and labeling of their genetic components.

1. Introduction

Infectious diseases constitute a severe threat to public health. For example, infection by food-borne pathogens causes 6–33 million cases of illness, including about 5000 deaths annually, in the United States alone (Hedberg, 1999; Kodell et al., 2002). For this reason, many researchers have tried to develop methods to detect the pathogenic infections quickly and accurately. Existing methods for multiplex pathogen detection include quantitative polymerase chain reaction (PCR) assay (Welti et al., 2003), immunoassay (Tok et al., 2006) and DNA microarray (Call et al., 2003; Eom et al., 2007; Hwang and Cha, 2008). Especially, the microarray system allows high-throughput and specific identification of pathogenic bacteria (Call, 2005; Szemes et al., 2005). In DNA microarray, genes encoding virulent factors, rDNA, or intergenic regions are usually amplified and fluorescently labeled during PCR, followed by hybridization with oligonucleotide microarrays specific to various pathogens. Up to 39 pathogenic bacteria have been successfully identified by this method (Yoo et al., 2009). Otherwise, 16S rRNAs are used as targets in DNA microarray, wherein amplification by PCR is not required due to the relatively high concentration of rRNA in the cell lysate (Chandler et al., 2003; Wang et al., 2004). However, a common problem of these microarray methods is long hybridization time, about 2–16 h. Therefore, a more rapid and efficient method for labeling and hybridization is needed.

Polydiacetylene (PDA) is considered to be an attractive material for the development of label-free chemosensors based on its unique properties, including a blue-to-red color change and self-fluorescence by external forces (Jelinek and Kolusheva, 2001). PDA vesicles consisting of diacetylene monomers, including 10, 12-pentacosadiynoic acid (PCDA), form stable, liposome-like bilayer structures in aqueous solution. Closely packed and well-ordered diacetylene monomers are polymerized by UV irradiation at 254 nm via 1,4-addition reaction, which changes triple bonds to ene-yne alternated polymer chain, resulting in the formation of the backbone (Charych, 1993; Jelinek and Kolusheva, 2007). The non-fluorescent polymerized PDA is blue in color, but it exhibits red color and fluorescence after being perturbed by external stimuli, such as heat, pH, organic solvents, mechanical stress, and ligand-receptor interactions (Ahn and Kim, 2008; Ahn et al., 2009). In general, there are two types of PDA sensors; liquid-phase sensors and immobilized sensors on solid substrates (Lee et al., 2010). Liquid-phase PDA sensors are very easy to detect, as their color transition is noticeable to the naked eye (Kolusheva et al., 2000; Reichert et al., 1995). However, they cannot easily detect multiplex samples and require a considerable amount of analytes. Therefore, microarray-type immobilized PDA vesicles were developed (Kim et al., 2003, 2005b). Immobilized PDA sensors, also called PDA chips, can be detected based on changes in fluorescence. They provide a few advantages, such as high portability, low sample consumption, and high integration capability for multiplex detection. To make PDA chips, immobilization of PDA vesicles is a crucial step. Immobilization with hydrophobic or charge interactions is plagued by the loss of PDA vesicles during washing steps and decreased efficiency due to insufficient immobilization (Park et al., 2009). For efficient immobilization, PCDA derivatives have been utilized to

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