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Detection of *Escherichia coli* O157:H7 with langasite pure shear horizontal surface acoustic wave sensors

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Abstract

The toxigenic *Escherichia coli* O157:H7 bacterium has been connected with hemorrhagic colitis and hemolytic uremic syndrome, which may be characterized by diarrhea, kidney failure and death. On average, O157:H7 causes 73,000 illnesses, 2100 hospitalizations and 60 deaths annually in the United States alone. There is the need for sensors capable of rapidly detecting dangerous microbes in food and water supplies to limit the exposure of human and animal populations. Previous work by the authors used shear horizontal surface acoustic wave (SH SAW) devices fabricated on langasite (LGS) Euler angles (0°, 22°, 90°) to successfully detect macromolecular protein assemblies. The devices also demonstrated favorable temperature stability, biocompatibility and low attenuation in liquid environments, suggesting their applicability to bacterial detection. In this paper, a biosensor test setup utilizing a small volume fluid injection system, stable temperature control and high frequency phase measurement was applied to validate LGS SH SAW biosensors for bacterial detection. The LGS SH SAW delay lines were fabricated and derivatized with a rabbit polyclonal IgG antibody, which selectively binds to *E. coli* O157:H7, in this case a non-toxigenic test strain. To quantify the effect of non-specific binding (negative control), an antibody directed against the trinitrophenyl hapten (TNP) was used as a binding layer. Test *E. coli* bacteria were cultured, fixed with formaldehyde, stained with cell-permeant nucleic acid stain, suspended in phosphate buffered saline and applied to the antibody-coated sensing surfaces. The biosensor transmission coefficient phase was monitored using a network analyzer. Phase responses of about 14° were measured for the *E. coli* detection, as compared to 2° due to non-specific anti-TNP binding. A 30:1 preference for *E. coli* binding to the anti-O157:H7 layer when compared to the anti-TNP layer was observed with fluorescence microscopy, thus confirming the selectivity of the antibody surface to *E. coli*.

Keywords: SAW; Langasite; *E. coli*; Antibodies; Immunosensor

1. Introduction

Dissemination of harmful bacteria in food or water supplies as a result of accidents, pollution or terrorist activity can produce serious consequences in the form of economic losses and human suffering. Sensitive and selective sensors capable of rapidly and accurately detecting minute quantities of pathogens are urgently required. The need for real-time detection of biological and biochemical analytes is a major driving force behind the development of novel biosensor technology (Drell and Sofaer, 1999; Gizeli and Lowe, 2002; Su and Yanbin, 2004).

A pathogenic strain of the normally benign *E. coli* bacterium is the target of this work. The O157:H7 *Escherichia coli* (*E. coli*) bacterium has been linked to hemorrhagic colitis and hemolytic uremic syndrome (Rangel et al., 2005). These illnesses may cause diarrhea, kidney failure, seizure, stroke and death. Illness due to *E. coli* is often misdiagnosed and commonly results in invasive and expensive medical tests before it is correctly diagnosed. Primary sources for exposure to O157:H7 are ground beef, unpasteurized milk, fruit, vegetables and unchlorinated water (Rangel et al., 2005).

Traditional methods in use for detection of *E. coli* involve standard clinical microbiological assays which are characterized by long incubation times on the order of 24–72 h (Baron and Finegold, 1990; Ivnitski et al., 1999). These methods generally entail sample collection and incubation at a local healthcare facility followed by transfer of the prepared sample to a

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centralized laboratory where apparatus and expertise for performing such tests have been concentrated. Tests for identification of pathogens often employ visual inspection with optical microscopy, flow cytometry, redox reactions, ultrasound techniques, gas phase chromatography, infrared spectroscopy and polymerase chain reaction (Ivnitski et al., 1999).

More recently biosensor techniques have been explored for microbial detection, in particular for use in the detection of pathogenic bacteria. Both indirect methods, which use labels or indirect methods as sensing mechanisms, and direct methods, which directly detect the target measurands, are under intense research and development (Ivnitski et al., 1999; Abdel-Hamid et al., 1999; Ruan et al., 2003). For instance, indirect methods are those which rely on the use of fluorescence labels, microbial metabolism, magnetoelastic immunosensors and electrochemical immunodetection. Examples of direct methods are: optical detection, bioluminescence, impedance measurement and acoustic wave detection for direct measurement of a physical property change due to an analyte (Ivnitski et al., 1999; Gizeli and Lowe, 2002). While indirect methods provide good sensitivity, direct methods are an alternative to indirect methods, which allow label-free detection of cells and tissues and therefore decrease the measurement complexity.

Examples of acoustic wave bacterial sensor platforms mentioned in the literature involve the use of commercially available quartz crystal microbalance (QCM) devices, which operate based on bulk acoustic wave resonation between smooth surfaces of a thin, piezoelectric, quartz wafer (Suleiman and Guilbault, 1994; Ivnitski et al., 1999; Deisingh and Thompson, 2002; Su and Yanbin, 2004). The thickness shear mode (TSM) on AT cut quartz is utilized in most QCM biosensors for mass detection, due to the low attenuation the mode exhibits in the presence of liquids.

Surface acoustic wave (SAW) devices are an alternative acoustic wave platform to the QCM, with the possibility of integration into array systems due to miniaturization, and potential increased sensitivity due to the surface guided nature of the wave and a higher operating frequency (Ballantine et al., 1997; Freudenberg et al., 2001; Gizeli and Lowe, 2002). These devices can be fabricated inexpensively in large quantities, utilizing single-step photolithographic processes, widely used in the semiconductor industry. The SAW devices can be configured as resonators or delay lines, with the maximum operating frequency constrained by the minimum achievable line-width of the photolithographic process, given a SAW velocity for a specific material and propagation direction.

For a SAW device to operate as a biosensor platform in liquid phase, the shear horizontal (SH) SAW mode is selected. Rayleigh or general polarized SAW modes excite longitudinal waves in the liquid, which results in significant SAW device losses (>40 dB), unusable in liquid media and therefore impractical as a biosensor (Calabrese et al., 1987; Kondoh and Shiokawa, 1993; Andle and Vetelino, 1995; Pereira da Cunha et al., 2002).

Three different techniques are listed in the literature for the detection of bacteria using bulk and surface acoustic wave devices: flow-through, dip-and-dry and immersion (Cunningham, 1998; Ivnitski et al., 1999; Gizeli and Lowe, 2002; Su and Yanbin, 2004). In flow-through experiments, bacteria in a buffer solution are allowed to flow over the sensing surface. In the dip-and-dry tests, the detecting surface is dried after it has been exposed to the bacteria. By allowing attached bacteria to dry onto the sensing surface, mechanical coupling between the sensor and the analyte bacteria is increased, which is advantageous in increasing the device's sensitivity. Finally, in immersion tests, the entire sensor device is immersed in a buffer solution to establish a baseline response, followed by submersion in the suspension of the target analyte bacteria.

SH SAW devices using quartz (Howe and Geoffrey, 2000) and pseudo-SAW (PSAW) devices using $LiTaO_3$ (Su and Yanbin, 2004) have been employed as immunosensors for bacterial detection. The SH SAW mode on quartz suffers from low electromechanical coupling coefficients, high penetration depth, and low dielectric permittivity with respect to the liquid media. The PSAW mode on $LiTaO_3$ suffers from extra attenuation in liquids due to the intrinsic associated vertical particle displacement component, and the attenuation of the acoustic wave due to the excitation of the BAW mode into the bulk of the crystal.

The langasite family of crystals (LGX), which includes langasite (LGS, $La_3Ga_5SiO_{14}$), langanite (LGN, $La_3Ga_{5.5}Nb_{0.5}O_{14}$) and langatate (LGT, $La_3Ga_{5.5}Ta_{0.5}O_{14}$), has been suggested as a possible alternative for SAW devices operating in liquids (Pereira da Cunha et al., 2002). Among the promising characteristics of these crystals applicable to liquid sensing, one can list: (i) the existence of pure SH guided SAW modes along Euler angles (0°, θ, 90°); (ii) a shallow acoustic penetration depth, in which most of the energy in the wave is concentrated at the surface, thus favoring sensitive SH SAW sensor response; (iii) the existence of SH SAW orientations with electromechanical coupling up to 10 times higher than the equivalent SH SAW 35.8°-Y rotated quartz, along Euler angles (0°, −54.2°, 90°); (iv) the occurrence of zero temperature coefficient of delay (TCD) for the SH SAW modes; (v) high values of dielectric permittivity, further confining the electrical fields in the piezoelectric crystal and thus improving transduction of the wave in the presence of high dielectric media such as water and (vi) a low SH SAW phase velocity, 50% below that of 35.8° Y rotated quartz, which favors smaller sensors.

Experimental SH SAW devices have been designed, fabricated, and tested along LGT and LGS orientations in (Pereira da Cunha et al., 2002) and (Berkenpas et al., 2004), verifying the properties previously listed and the suitability of LGS for operation in liquids. LGS operation along the propagation direction Euler angles (0°, 22°, 90°) presents (Berkenpas et al., 2004): (1) relatively strong piezoelectric coupling (0.4%); (2) reduced additional attenuation (6 dB) due to liquid loading or lossy material, such as liquid chamber rubber, at the surface; (3) reduced temperature dependence, with zero temperature coefficient of frequency (TCF) or turn over temperature identified at 20 °C, and a fractional frequency variation, $\Delta f/f_o$, of 2.5 ppm over 5 °C around the turn over temperature and (4) suitability for biological detection, indicated by the fabricated and flow-through tested LGS SH SAW devices, which produced measurable transmission phase variations due to antibody binding on the sensor surface.