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# Foodborne pathogen detection using surface acoustic wave biosensors: a review

Yujia Zeng,[a] Rui Yuan,[a] Hao Fu,[a] Zhangliang Xu *[a] and Song Wei[b]

This paper summarizes several attractive surface acoustic wave (SAW) biosensors, including Love-wave sensors, dual-channel SAW sensors, langasite SAW sensors, and SAW syringe filters. SAW sensors with different piezoelectric materials and high-frequency SAW sensors used for identifying the food pathogenic bacteria *Escherichia coli* (*E. coli*) are discussed together with the examples of methods based on such sensing technology that have been effectively utilized in diagnostics and epidemiological research. This review also emphasizes some of the limitations of using these biosensors, which have prompted the increased need for more rapid, sensitive, selective, portable, power-efficient, and low-cost methods for detecting these pathogens. It is envisioned that SAW devices will have remarkable significance in the future.

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## 1. Introduction

Foodborne illnesses are a common and growing concern in both developed and developing nations, making food safety an increasingly essential public health issue.[1,2] Foodborne pathogens (Table 1) are particularly dangerous for children, with 220 million becoming sick and 96 000 dying each year, and the yearly cost of these illnesses is US $5–6 billion.[3] The danger of infection with the well-known food pathogen *Escherichia coli* (*E. coli*) has increased over time. *E. coli* is a Gram-negative bacterium of a rare serotype, belonging to the family Enterobacteriaceae, and is typically found residing in the intestines of healthy humans and animals.[4–7] It was first isolated in 1885 from children's feces by the German bacteriologist Theodor Escherich. The bacteria measure approximately 2–6 μm in length and around 1–1.5 μm in diameter.[8–10] *E. coli*, a member of the coliform bacterial group, is predominantly responsible for the majority of urinary tract infections. 'Coliforms' belong to four species: *E. coli* (human intestines), *Citrobacter* (bacteremia, brain abscesses, and pneumonia), *Enterobacter* (urinary tract infections, meningitis, and sinusitis), and *Klebsiella* (gastrointestinal tracts of animals). These bacteria produce a powerful toxin known as Shiga toxin. This toxin attacks small blood vessels and can cause serious damage to intestinal cells, resulting in hemorrhagic colitis (HUS), bloody diarrhea and severe abdominal pain, stomach cramps, vomiting, dehydration, seizures, stroke, and kidney failure,[11–13] and is generally found to be responsible for the majority (20%) of outbreaks globally.[3,14] The presence of higher than 1 CFU *E. coli* O157 : H7 in 25 g of food has been considered a dangerous level.[15,16] Although the majority of *E. coli* bacteria are not harmful, a few can cause serious food poisoning.

Table 1 describes different types of foodborne pathogens and their sources, yearly mortality rate, effects on the human body, and different detection methods. The satisfactory microbiological quality for *E. coli* O157 is <20 CFU $g^{-1}$, with the acceptable range being 20–<100 CFU $g^{-1}$,[3] and the time taken for the occurrence of diarrhea can range from 1 to 8 days. Primary sources of *E. coli* exposure include contaminated water and food, particularly raw vegetables, undercooked ground beef (hamburger), unpasteurized milk, and fruit juice.[9,11,23,24] Additionally, the utilization of shared facilities facilitates *E. coli* transmission, thereby increasing the likelihood of an outbreak. To minimize the risk of exposure among human and animal populations, it is essential to develop sensors that can rapidly detect hazardous *E. coli* in food and water supplies.

As a result, several studies have been published to detect *E. coli* contamination in food and water, and have proposed various methods and sensors, including surface plasmon resonance biosensor,[25] magnetoelastic immunosensor,[14] polymerase chain reaction (PCR),[26] enzyme-linked immunosorbent assay (ELISA),[27] piezoelectric immunosensor,[28] and quartz crystal microbalance (QCM).[29] The majority of these detection techniques are based on optical, electronic, and electrochemical methods, which offer a rapid response and ease of operation.[30] However, these methods are specific and sensitive, and also have limitations related to their high costs, time-consuming procedures (48–72 h), need for expensive laboratory facilities and trained personnel, and complex procedures, which obstruct the widespread use of these technologies for diagnosis.[11] To overcome such limitations and replace these old

[a]*School of Electronic Information Engineering, China West Normal University, Nanchong 637009, China. E-mail: xuzl906@163.com*
[b]*School of Mechanical and Electrical Engineering Guilin University of Electronic Technology, Guilin 541000, China*

Table 1 Common global diseases caused by bacterial infection, with the main sources of infection, key effects, annual deaths, and common detection methods

| Pathogen | Source | Effects | Annual deaths | Detection method | Reference |
|---|---|---|---|---|---|
| *E. coli* O157 : H7 | Raw vegetables, undercooked beef unpasteurized milk, fruit juice | Diarrhea, stomach pain, hemorrhagic colitis, and HUS | 200 000 | Conventional methods, ELISA PCR, biosensors, *etc.* | 3 |
| *Salmonella* | Raw and undercooked egg, fruits and vegetables | Diarrhea, fever, and stomach cramps | 155 000 | RT-PCR, SPR, QCM, impedimetry, *etc.* | 17<br>18 |
| *Shigella* | Salads and unclean water | Diarrhea, fever, and stomach cramps | 1.1 million | PCR, rep-PCR, culture methods, immunological methods *etc.* | 19<br>17 |
| *Clostridium botulinum* | Meat, poultry, cooked dried beans and gravies | Diarrhea and stomach cramps | — | PCR, nested PCR, real-time PCR | 20 |
| *Norovirus* | Eating food or drinking liquids contaminated by an infected person | Vomiting, diarrhea, and stomach cramps | 200 000 | Real-time PCR | 10 |
| *Staphylococcus aureus* | Cooked ham, salads, and bakery products | High fever, nausea, and vomiting | 19 832 | Conventional methods, molecular biological methods, optical immunoassays | 9 |
| *Bacillus cereus* | Raw plant foods, such as rice, potatoes, peas, beans, and spices | Intestinal illnesses with nausea, vomiting, and diarrhea | — | Immunosensors and DNA biosensors *etc.* | 21 |
| *Listeria monocytogenes* | Raw, unpasteurized milks and cheeses, ice cream, raw or processed vegetables, fruits | Fever, muscle aches, headache | 260 | Conventional culture methods, PCR *etc.* | 22 |

procedures, the use of surface acoustic wave (SAW) devices for both sensing and actuation is proposed on a single platform to create rapid, real-time, cost-effective, and labor-efficient techniques for the detection of *E. coli* bacteria.

For sensor applications, the quartz crystal microbalance (QCM) is a simple and commonly utilized acoustic transducer, with minimal manufacturing costs. However, because of its low resonance frequency, it has limited sensitivity. On the other hand, surface acoustic wave (SAW) sensors are well-known and frequently employed in gas and liquid media with a variety of commercial applications. SAW sensors are more sensitive than QCM sensors (Table 2) because waves in QCM sensors must propagate through the whole piezoelectric substrate, but waves in SAW sensors only have to propagate through a guiding layer at the surface, resulting in more concentrated energy. SAW sensors also exhibit greater sensitivity compared to QCM sensors, as indicated in Table 2. SAW sensors exhibit superior sensitivity due to the higher operating frequency and concentration of acoustic wave energy at the surface. This characteristic renders SAW sensors more advantageous compared to QCM sensors. SAW sensors are capable of functioning at elevated frequencies, ranging from several hundred megahertz (MHz) to gigahertz (GHz). In contrast, QCM sensors are limited to operating within the frequency range 5–30 MHz. Owing to the aforementioned advantages, research on SAW sensors in the bio-analytical domain, particularly for applications such as *E. coli* detection, is experiencing rapid growth. SAW sensors are capable of detecting changes in mass or viscosity through wave velocity attenuation,[29] offering high sensitivity and enhanced compatibility with complementary metal-oxide semiconductor (CMOS) technology.[31] The detection limit of SAW sensors is approximately in the picograms range ($10^{-12}$ g).[32] Additionally, they also provide a wide array of functionalities within a compact and durable package.[33]

Table 2 shows that SAW sensors offer superior performance or more advantages than QCM sensors. SAW-based biosensors also offer more benefits than other types of biosensors, such as optical and mass-based biosensors. SAW technology plays an important role in many physical, chemical, electrical engineering, and biological sensing applications.[34–37] SAW devices have been typically used in the Rayleigh and shear horizontal (SH) SAW propagation modes,[31] wherein Rayleigh SAW is suitable for gas-sensing applications, but not well suited to liquid sensing. This is because Rayleigh waves have both a surface-normal component as well as a surface-parallel component with respect to the propagation direction and hence can couple strongly with a liquid.[38] They can even work without batteries and can also operate in harsh environments.

In this review, we emphasize recent advances in the use of SAW sensors for detecting *E. coli* bacteria, including the Love-wave immunosensor, dual-channel SAW sensor, and SAW syringe filter. Both detection and separation/concentration techniques have advanced significantly in recent years. With a focus on distinct domains, this review examines the existing conventional approaches as well as developments in biosensor techniques for detecting *E. coli* bacterial infections.

Table 2 Comparison of SAW and QCM techniques

| Technical index | SAW | QCM |
|---|---|---|
| Acoustic wave type | Surface | Bulk |
| Sensitivity | High | Low |
| Frequency | 10 MHz to 3 GHz | 5–10 MHz |
| Dielectric constant | $LiNbO_3 = 29$, $LiTaO_3 = 43$ | 3.8 |
| Detection range | $10^{-12}$ g | ~1 Å to 1 μm or $10^{-9}$ g |
| Piezoelectric material | ST-cut quartz, $LiNbO_3$, $LiTaO_3$ | AT-cut quartz |
| Temperature range | 25 °C to 1000 °C | −190 °C to 125 °C |
| Working area | Liquids and gases | Liquids and gases |
| Cost | Low | High |
| Applications | Filters, cell monitoring and manipulation, signal processing units, sensors, and actuators | Detection of metals in vacuum, vapors, chemical analytes, biomolecules, and contaminants in the environment |

## 2. Working principle of SAW biosensors

SAW was first reported by Lord Rayleigh in 1885, and updated by White and Voltmer in 1965. A SAW element is made of a piezoelectric substrate, such as quartz ($SiO_2$), lithium niobate ($LiNbO_3$), lithium tantalite ($LiTaO_3$), zinc oxide (ZnO), or aluminum nitride (AlN),[39–41] and interdigitated transducers (IDTs). For applications involving biological detection, it is necessary to implement chemical and biological modifications within the detection region of the SAW sensor, as shown in Fig. 1. SAWs can be generated by applying an appropriate electric field to the IDTs. Under the dynamic change of the electrical input signal caused by electromechanical coupling, the electrical component creates mechanical energy as acoustic waves in the piezoelectric substrate.

In applications involving the detection of chemical and biological substances, SAW biosensors rely on mass loading, surface perturbation, chemisorption, and biological affinity to achieve a highly sensitive response to the surface of the substance under test. The signal generated by the reaction of the test sample with the biologically active substance is subsequently converted into an electrical signal output by the sensor. Biomolecules, including antibodies, enzymes, phages, and DNA probes, are coated on the surface of the SAW biosensor to capture specific target molecules, such as antigens, viruses, and DNA sequences. Upon binding the target biomolecule (bacteria or virus) to the specific recognition molecule on the biologically modified layer, the mass-loading-based effect brings about shifts in the SAW resonance frequency, phase, and other characteristics. By amplifying, receiving, transforming, and filtering these altered characteristic signals, a quantitative detection and analysis of the measured object can be achieved.

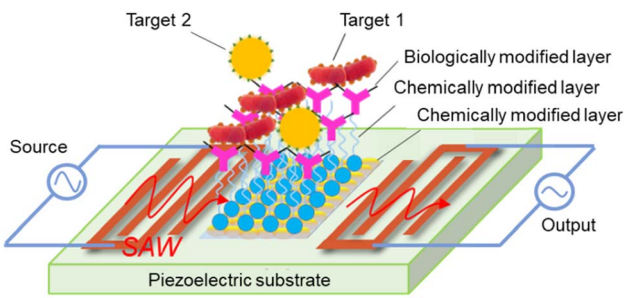


Fig. 1 Basic structure of a SAW biosensor.

The sensitive layer of SAW biosensors is the core recognition element of microorganisms, such as bacteria and viruses, which is used to deposit biological samples, but also comes into contact with corrosive environments, such as strong acids and alkalis, during the detection process. Therefore, the sensitive layer material should have good biocompatibility and certain corrosion resistance. The deposition of an Au film, oxide film (ZnO, $In_2O_3$, GO),[42–44] polymer film (PEI, PEN),[45,46] composite film ($ZnO–SnO_2$),[47] and other specialty film materials on the surface of the sensitive layer for chemical modification facilitates capture of the target chemical molecules and enhances the adhesion of biomolecules. Among these materials, Au is of particular interest to researchers due to its chemical stability and its capacity to form self-assembled molecular layers through the combination of a range of biomolecules with end-modified sulfhydryl groups.[48]

## 3. Applications of SAW biosensors for *E. coli* detection

Over the last several decades, SAW biosensor technology has been increasingly developed for the detection of foodborne pathogenic bacteria.[49] Numerous researchers have successfully developed methods to detect bacteria or microorganisms in water and food, owing to the technology's high stability, low limit of detection (LOD), low power consumption, flexible design, ease of miniaturization, and potential for integration into microfluidic devices.[11,39,50,51] It has also been applied for detecting microorganisms in real time because the sensors are relatively cost-effective, due to their small size, high sensitivity[11,41,45] and high electromechanical coupling coefficient. Table 3 shows a comparison of several SAW biosensors for *E. coli* detection. These SAW biosensors all demonstrate high electromechanical coupling performance and high sensitivity for *E. coli* detection.[33,52–54]

### 3.1 Love-wave sensors

A Love wave is a type of horizontally polarized shear wave that is produced at the surface boundary of an elastic medium as

Table 3 SAW biosensors based on different sensitive layers for the detection of *E. coli*

| Type of substrate | Wave mode | Dielectric constant | TCF (ppm per °C) | Velocity (m $s^{-1}$) | Detection limit or sensitivity | Reference |
|---|---|---|---|---|---|---|
| 64°YX $LiNbO_3$ | SH-SAW | 85.2 | 80 | 4742 | $1.8 \times 10^{-15}$ M | 55 |
| AlN | Love wave | 8.5 | 19 | 5700 | $6.54 \times 10^5$ CFU $mL^{-1}$ | 45 |
| $LiTaO_3$ | SH-SAW (LGS) | 470 | −33 | 4160 | $10^6$ cells per mL | 56 |
| $SiO_2$ | Love wave | 3.8 | — | — | $10^5$ cells per mL | 57 |

a result of multiple total reflections. The propagation characteristics are mainly determined by the shear modulus, whereby the amplitude decays exponentially along the depth direction, and along the surface with the regularity of $r^{-1/2}$. The existence of the Love wave was first predicted by the mathematician Augustus Love in 1911 in the context of seismic research.[58] Physical information about the base and the waveguide layer are conveyed by the Love wave throughout its propagation process. The mechanical properties of the material, comprising the waveguide layer, including the elastic modulus and density, exhibit a discernible pattern of change in their spatial distribution.[59,60] The presence of a low-velocity layer on a semi-infinite elastomer results in the accumulation of acoustic waves within the waveguide layer, due to the discrepancy in mechanical properties between the waveguide layer and the substrate. This phenomenon reduces the acoustic penetration of the substrate and consequently decelerates the propagation speed of the wave. The concentration of acoustic waves in the waveguide layer renders the device highly sensitive to alterations in surface mass loading, viscosity, and conductivity. Consequently, Love waves are frequently employed for sensing and detection in both gas-phase and liquid-phase media. Guided shear horizontal surface acoustic wave sensors are known as Love-wave sensors. Love-wave devices are excellent for real-time sensing applications. Love-mode SAW sensors, fortunately, have received a great deal of attention during the last two decades due to their high miniaturization and sensitivity, making them suitable for use in portable devices for point-of-care testing applications or for on-site measurements.[61] Also, some researchers successfully applied their Love-wave methods to detect harmful pathogenic microorganisms, such as *E. coli* bacteria.

**3.1.1 Working principle of Love-wave sensors.** In a Love-wave sensor, a pure shear horizontal wave is created depending on the crystallographic orientation of the quartz. IDTs on the quartz substrate create and receive an elastic wave in these sensors, which are based on a piezoelectric delay line. The active sensing layer is an important part of the Love-wave sensor because any changes or disturbances on its surface induces changes in the acoustic wave velocity or amplitude, and hence a frequency shift response. Any physicochemical disturbance on the sensor surface will change the wave velocity, which may be detected with high precision by frequency in an oscillator configuration, giving the device a high measurement resolution. The basic structure of a Love-wave surface acoustic wave sensor is presented in Fig. 2.

To get Love-wave modes, the shear velocity of the guiding layer must be lower than that of the substrate. The higher the

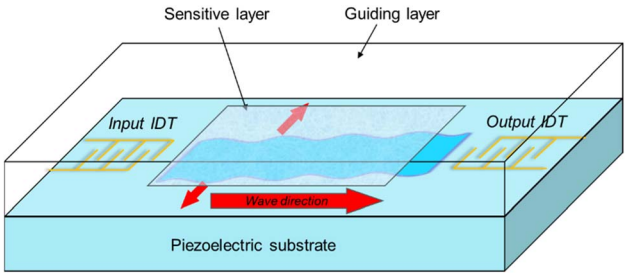


Fig. 2 Basic structure of a Love-wave surface acoustic wave sensor.

sensitivity, the bigger the difference in velocities. The sensitivity to surface mass loading for the acoustic sensor can be defined as the relative change in oscillation frequency due to mass loading on the surface,[62] with the typical operation frequencies of such sensors being between 80–300 MHz.[63] If the piezoelectric material is put on top of the piezoelectric substrate and over the layer with a lower shear velocity, Love waves propagate near its surface, which promote shear horizontal (SH) waves. The wave velocity and amplitude are highly influenced by changes in the medium near the surface.

**3.1.2 *E. coli* detection using a Love-wave immunosensor.** Several writers have investigated Love waves in recent years. Love-wave devices appear to be powerful devices for biodetection.[64] The first Love-wave sensor for biochemical sensing was reported in 1992 by Kovacs *et al.*[65] Then, Harding *et al.*[66] used a Love-wave acoustic device to detect real-time antigen–antibody (Fig. 3(a)) interactions in liquid media. In 2000, Howe and Harding[57] used a dual-channel Love-wave device as a biosensor to simultaneously detect *Legionella* and *E. coli*. They presented a unique methodology for coating bacteria on the sensor surface and their quantitative results were $10^6$ cells per mL within 3 h. In 2003, Tamarin *et al.*[67] designed a Love-wave immunosensor as a model for virus or bacteria detection in liquids. In 2007, Moll *et al.*[68] developed an innovative method for the detection of *E. coli* employing an LW immunosensor. They divided their method into two steps: in the first step, goat anti-mouse antibodies (GAM) were grafted onto the sensor surface, and in the second step, *E. coli* bacteria were mixed with anti-*E. coli* antibodies and introduced onto the sensor. Later, antibody–antigen interactions were used to detect biological species, like bacteria, viruses, and toxins.[69] Finally, their detection threshold was $10^6$ bacteria per mL. More recently, the same authors[70] described a multipurpose Love-wave immunosensor for the detection of bacteria, viruses, and proteins and they successfully applied this sensor to detect bacteriophages

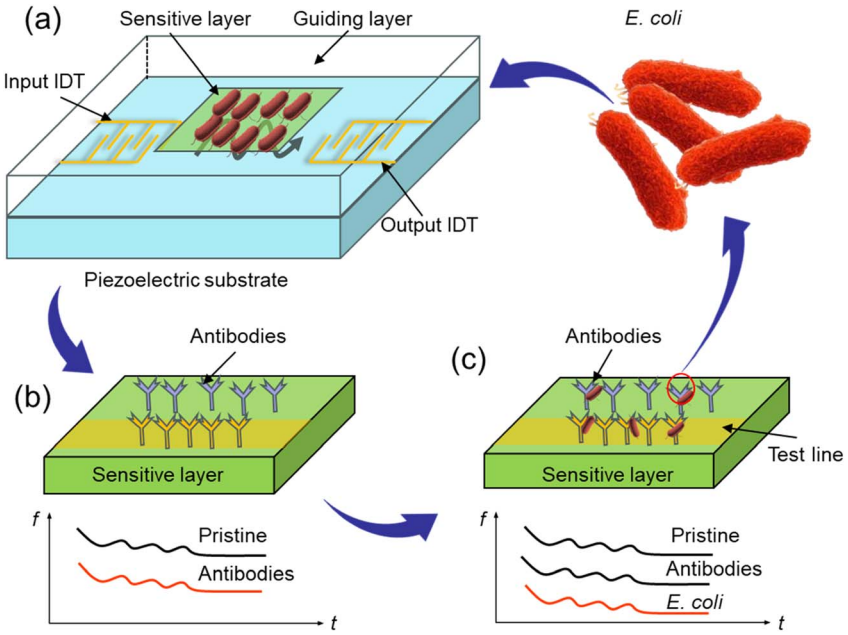


Fig. 3 Configuration of antibody binding onto the sensor surface. (a) SAW biosensors construction, (b) antibody immobilization, (c) *E. coli* adsorption.

and proteins down to 4 ng $mm^{-2}$. They also successfully demonstrated that *E. coli* bacteria could be identified up to 5.0 $\times$ $10^5$ cells in a 500 μL chamber. Furthermore, the entire bacterial detection process could be completed in less than 1 h, exhibiting high specificity and reproducibility.

In other work, a $SiO_2$ thin film and 3-glycidoxypropyltriethoxy silane (GPTS) were decorated on the surface of a SAW device, with GPTS (Fig. 3(b)) used to covalently bond to the $SiO_2$ substrates by a reaction between the silanol groups of the surface and alkoxysilanes.[71,72] Then the antibodies could be covalently linked to the modified surface by a reaction between the epoxy group of GPTS and the amino groups of the antibodies.[73] The GPTS coupling agent (Fig. 3(c)) molecule was used to graft the antibody onto the $SiO_2$ sensor surface, and the grafting frequency signal of the antibodies was monitored. Wave propagation was disrupted when the sensitive layer captured the species, resulting in a drop in wave propagation due to the mass addition on the sensor's surface (mass-loading effect). As a consequence, any biological species with enough total mass immobilized onto the sensor surface to alter the acoustic wave can be detected. Finally, the detection threshold

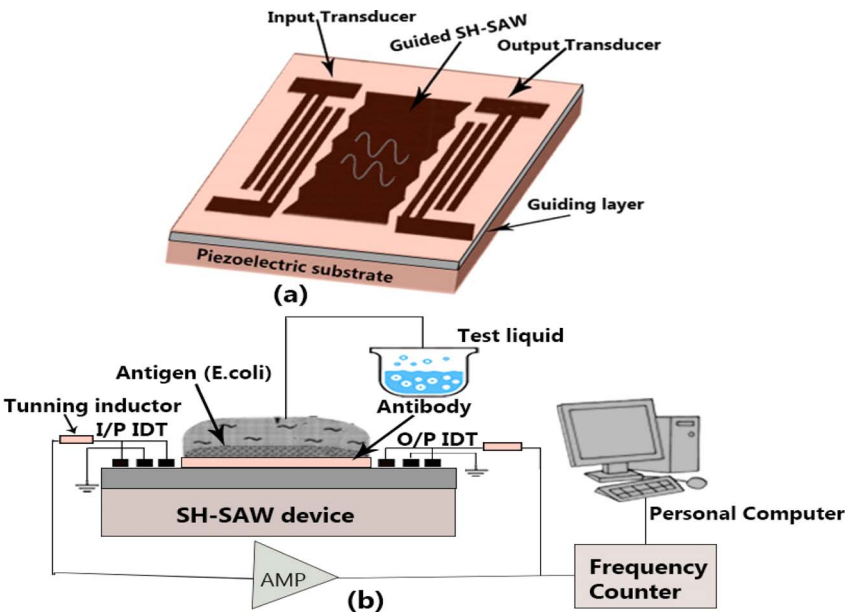


Fig. 4 Schematic representation of the SH-SAW delay-line.[91]

was found to be $10^6$ bacteria per mL in a 500 μL chamber at a temperature of 37 °C in under 1 h.[68]

The antibody could be bound to the $SiO_2$ surface by adsorption or by a covalent link using a coupling agent. The Love-wave sensor can detect targets in the nano-molar range and has been utilized for monitoring antibody–antigen immunoreactions in aqueous solutions in real time.[74] These sensors are the most recently developed devices designed for integration in "lab-on-a-chip" systems.[75] However, further study is needed to improve the sensor's sensitivity before it can be used to detect individual microbes. Hopefully, SAW sensors based on Love-wave immunosensors will be widely applied in the future for a variety of applications, especially for pathogen detection.

### 3.2 SH-SAW biosensors with different piezoelectric materials

Shear horizontal-SAW (SH-SAW) sensors have been used in a variety of applications, including biosensors, taste sensors, and liquid- and gas-media measurements. In liquids, the SH-SAW device maintains high sensitivity and is suitable for "real-time" sensing and detection. This was the first leaky wave sensor that was only partially contained on the surface. Shear horizontal mode surface acoustic waves have a unique characteristic of complete reflection at the free edges of the substrate[76] and can allow detecting complex permittivity with high sensitivity in liquids.[77]

In 1987, Moriizumi *et al.*[78] described the first biosensor applications of SH-SAW transducers in a liquid medium. Deobagkar *et al.*[79] developed a SH-SAW immunosensor for *E. coli* detection in water; whereby, using a 87.7 MHz oscillator, they achieved a detection range of 0.4–100 cells per L, yielding frequency changes of 1.5–5.8 kHz. As a result, the device was sensitive enough to identify *E. coli* at levels that are dangerous to human health. Länge *et al.*[80] presented a new approach that involved integrating a SH-SAW biosensor in a microfluidic polymer chip. In 2008, Bisoffi *et al.*[81] developed an SH-SAW biosensor that combined the sensitivity of 325 MHz SAW with the specificity given by antibodies for the detection of viral bioagents. They used a $LiTaO_3$-based SAW transducer with a $SiO_2$ waveguide sensor platform to capture adsorb antibodies. Ten *et al.*[11] also described an SH-SAW biosensor with a $SiO_2$ guiding layer to detect *E. coli* bacteria. They employed a 22-mers DNA sequence from *E. coli* O157 : H7 as an amine-terminated probe ssDNA that was chemically functionalized and immobilized on the thin-film sensing region [(CHO–$(CH_2)_3$–CHO) and (APTES, $NH_2$–$(CH_2)_3$–$Si(OC_2H_5)_3$)]. Their sensor performed well with a particular oligonucleotide target, with a sensitivity of 0.6439 nm/0.1 kHz and a detection limit down to 1.8 femto-molar ($1.8 \times 10^{-15}$ M).

For the detection of *E. coli* bacteria, Lamanna *et al.*[45] developed the first conformable SAW biosensor using polyethylene naphthalate (PEN). On a PEN substrate, they created a SAW immunosensor with a piezoelectric AlN material. They also performed detection experiments with a comparable SAW immunosensor developed on a silicon substrate to evaluate and compare the performance of their device. Their investigations

Table 4 Several types of piezoelectric materials used in SH-SAW sensors and their advantages

| Materials | Orientation | Frequency range (MHz) | Coupling coefficient, $K^2$ (%) | Dielectric constant | TCF [ppm per °C] | Advantage | Reference |
|---|---|---|---|---|---|---|---|
| 36° YX $LiTaO_3$ | *x*-axis | 100–150 | 5–6.6 | 43 | 32 | Reduces the TCF, label-free detection, minimal energy loss, low signal loss, reproducibility | 84 and 85 |
| 64° YX $LiNbO_3$ | *x*-axis | ~100 to ~450 | 46.4 | 85.2 | ~80 | Low cost, low power consumption, wide application, suitable for fluid loading | 86 and 87 |
| 36° YX $LiNbO_3$ | | | | | | | |
| Quartz | *z*-axis | 134–570 | 0.1–0.2 | 3.8 | 0 | High sensitivity and temperature stability, low insertion loss | 82 and 84 |
| ZnO | *c*-axis | 153 | 1.5–1.7 | 8.66 | ~−60 | Best microfluidic performance, simple operation, precision, low cost, fast response, and reduced reagent requirements | 82, 85 and 88 |
| All | | 520 | 3.1–8 | 8.5 | 19 | | |

determined that their PEN-based SAW biosensor had a limit of detection of $6.54 \times 10^5$ CFU $mL^{-1}$, which was superior to the silicon-based SAW biosensor's limit of detection of $1.04 \times 10^5$ CFU $mL^{-1}$.

Fig. 4 illustrates a special delay line type SH-SAW biosensor. The SH-SAW is directly generated by IDT excitation and propagated in a multi-layer structure. Table 4 lists several SH-SAW mode sensors based on different piezoelectric materials. Piezoelectric materials with special crystal cutting angles, such as 36° YX $LiTaO_3$, 64° YX $LiNbO_3$, and ST-90° X quartz, are often used as piezoelectric substrates for the direct generation and propagation of SH-SAWs in liquid media.[82] Bergaoui *et al.*[83] reported antigen–antibody recognition using a $LiTaO_3$ SH-SAW sensor. They showed that grafting numerous molecular layers onto the SAW sensing region allowed streptavidin–anti-streptavidin identification. The antigen–antibody reactions with the sensing region of the SH-SAW biosensor device were used to evaluate its sensing qualities.

Acoustic wave technologies also use piezoelectric thin-film materials, including ZnO, and AlN. Among these thin-film materials, AlN thin films have been widely investigated due to their unique large wave velocity[89] and higher frequency.

Before bio-molecular immobilization, piezoelectric materials create a waveguide around both transducers and reduce the attenuation. Both the particle displacement and electrical potential interact with the liquid when the surface is free and electrically active. This is an electrical interaction with the liquid that changes the velocity and/or attenuation of SH-SAW propagation and is used in liquid sensing. The SH-SAW propagating characteristics are modified by the nature and conditions of the molecules and liquid surfaces loaded on the sensing area.[90] The frequency changes in response to varying amounts of *E. coli* O157 : H7 have been comprehensively explored with this sensor.

The main advantage of the SH-SAW sensor is that it can detect various properties of an adjacent liquid without any membrane[92] with a high electromechanical coupling coefficient ($K^2 = 5\%$).[93] However, it has some drawbacks; for instance, both $LiNbO_3$ and $LiTaO_3$ are piezoelectric materials and are dependent on the temperature, limiting the operation at elevated temperatures.[94] Also, the IDTs are generally made of low-cost aluminum,[80] which means that their lives in aqueous conditions are limited because of corrosion. As a result, more layers of protection are necessary.

### 3.3 Langasite-based SH-SAW sensors

This section describes a novel LGS SH-SAW sensor for the detection of *E. coli* bacteria. Langasite (Fig. 5) is a novel piezoelectric material that can be used to make surface acoustic wave devices. It offers a new opportunity for high-temperature sensors (up to 1470 °C) with a reported sensitivity of 749 Hz $ng^{-1}$ $m^{-2}$.[95] The langasite family of crystals (LGX), including langasite (LGS), langanite (LGN), and langatate (LGT), belong to the symmetry class 32. A delay line was designed and fabricated on a (0°, 22°, 90°) with a high dielectric constant.[96] Due to the delay lines, langasite SH-SAW (LGS) sensors may be used to detect bacteria. They match the requirements for sensitivity,

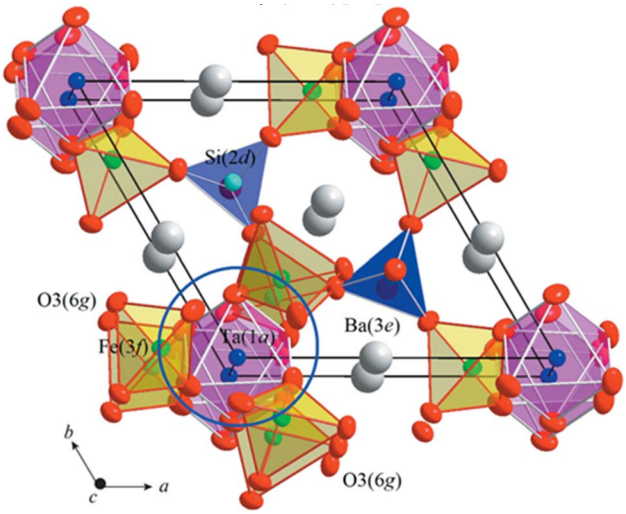


Fig. 5 Crystal structure of langasite.[97]

temperature stability, compatibility with biochemically produced recognition receptors, and low attenuation in liquid environments. They also exhibit a zero-temperature coefficient of frequency (TCF).[85]

Several researchers have investigated langasite-based SAW sensors. In 2005, Pollard and Pereira Da Cunha,[98] using finite thickness gratings, reported an improved pure SH-SAW transduction efficiency on LGS. Ayala *et al.*[99] presented LGS temperature stability for SH-SAW sensor applications. No one had published research on detecting bacteria using LGS-SH-SAW sensors before Berkanpas. Berkenpas and colleagues investigated for a long time and published several articles based on LGS-SAW sensing, including for the detection of *E. coli* bacteria. In 2003, they effectively detected macromolecular protein assemblies using an SH-SAW transducer based on langasite (LGS) crystals.[96] They reported that the device had excellent temperature stability, biocompatibility, and minimal attenuation in liquid environments. At the same time, they also suggested that it could be used to identify bacteria. In the same year, they developed an LGS SH-SAW[100] sensor that could detect microbial pathogen-derived biomolecules in aqueous solutions, such as nucleic acids. They used a liquid flow-through technique to make a sensor capable of recognizing nucleic acids. Later in 2006, the same authors[56] developed the LGS SH-SAW biosensor for *E. coli* O157 : H7 detection. They used a biotinylated polyclonal rabbit polyclonal immunoglobulin G (IgG) antibody (Fig. 6(b)) directed against *E. coli* to bind to a NeutrAvidinTM SAM-functionalized gold surface to derive LGS SH-SAW delay lines. In their experiment, they used two different methods: liquid-phase flow-through and dip-and-dry methods. Due to the relatively large distance of the *E. coli* from the sensitive LGS SH-SAW surface with the viscous decay length, the flow-through technique generated a lower (0.1–0.7) variance in the $S_{21}$ response. Also, a large and detectable change in the $S_{21}$ response was achieved using the dip-and-dry method (Fig. 6(a)), in which *E. coli* were selectively bonded and then dried onto the surface of the LGS SH-SAW delay line.

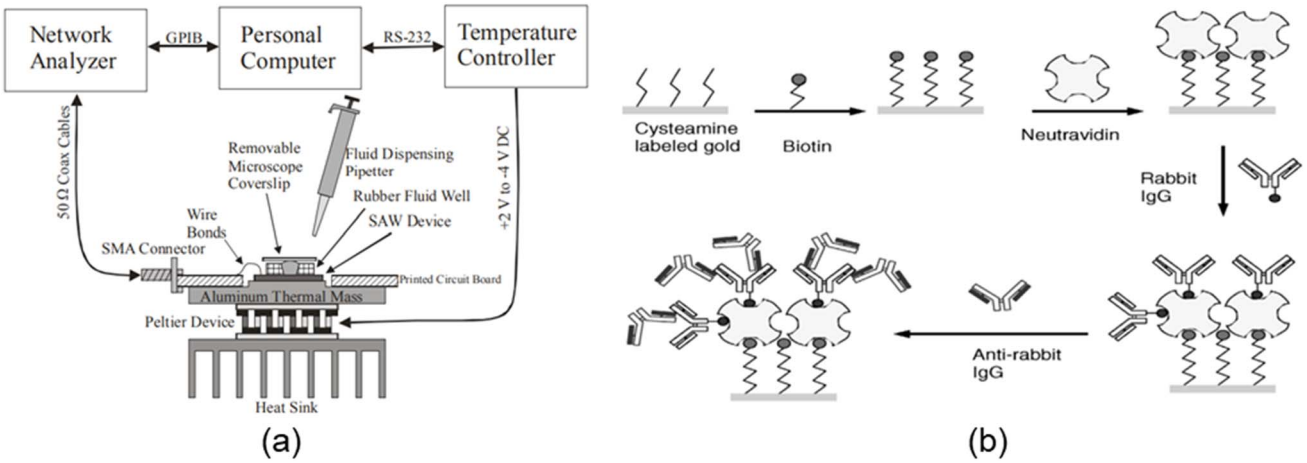


Fig. 6 Schematic structure of (a) dip-and-dry test[101] and (b) immunochemical reactions and biomolecule additions on the LGS SH-SAW sensor with phase changes.[102]

To evaluate the SH-SAW device's performance as a biomolecule binding sensor, a series of immunochemical reactions were generated on its surface and then measurement was made of the quantity of antigen bound onto surface-immobilized antibodies, which may bind specific antigens, to determine the concentration of target specimens. *E. coli* were selectively bound and then dried onto the LGS SH-SAW delay line's surface. After an initial drop, phase $S_{21}$ increased slightly as the *E. coli* dried on the sensor surface. The dehydration of the *E. coli* reduced their mass, which reduced the total mass on the sensor surface, resulting in a small recovery of the phase response. Larger phase variations were observed when larger amounts of surface-bound *E. coli* were present. Dry *E. coli* attachment to the sensing zone increased the surface mass and delayed the SH-SAW, resulting in a downward phase shift. All the measurements performed well at 25 °C for this procedure (dip and dry). Finally, the detection limit was estimated at $\sim 10^6$ cells per mL based on a measured $<S_{21}$ noise of 0.05°. Although this sensor has numerous benefits, we hope the sensitivity of LGS SH-SAW biosensors will be improved in the future by optimizing both the bio-receptive layer and the LGS SH-SAW platform modeling and design.

### 3.4 SAW impedance sensor

The impedance method is one of the earliest electrical methodologies, and it is based on the measurement of changes in a medium's electrical characteristics as a result of microbial population development. The impedance-based SAW sensor is a new type of SAW device that is good for remote sensing and multi-sensor identification,[103] and is capable of detecting bacteria.

This subsection introduces a novel SAW impendence sensor that can detect the density of *E. coli* in environmental water sources. Unique novel SAW impedance biosensors have been reported for bacteria detection based on impedance measurements of bacterial cells' electrical characteristics when they are connected with electrodes. The SAW sensor converts the impedance signal from the interface of the electrodes in the bacterial growth fluid into a frequency signal. This sensor responds to changes in the capacitance and conductance of the solution between the two electrodes. Generally, in a medium inoculated with the testing bacteria, a pair of metal electrodes are submerged. Antibodies specific to the target bacterial cells are immobilized on the electrode surface to produce an impedance biosensor for bacterial cell identification. The impedance changes in the medium produced by bacterial metabolism are monitored in real-time. Various researchers have investigated SAW-based impedance sensors, including Wang *et al.*,[104] who for the first time used a SAW-based impedance sensor for acid phosphatase detection and micro-analysis in 1998. In 2004, Karilainen *et al.*[105] provided a SAW biosensor for electrocardiogram (ECG) and other bio-potential monitoring. Nguyen *et al.*[106] fabricated a SAW impedance sensor for measuring the input impedance $S_{11}$ in a microfluidic approach. Yang and Bashir[107] described the principles of impedance biosensors for foodborne pathogenic bacteria detection and their detection limit was $\sim 10^4$ CFU $mL^{-1}$ for *E. coli* in under 2 h. However, to the best of our knowledge, there is no reported research on the use of a SAW-based impedance sensor to detect harmful microorganisms, especially *E. coli*.

However, Chang and colleagues[108] used a SAW-based impendence syringe filter (Fig. 7) to successfully detect *E. coli* bacteria in water sources in 2012. They modified a SAW sensor by adding a syringe filter with a Teflon cover, which allowed the $CO_2$ gas produced by bacteria to flow upward and collect in the syringe filter's headspace. The frequency of the SAW sensor changed in response to the presence of $CO_2$ gas around the electrode surfaces, which was created by coliform growth. The detection limit was $10^2$–$10^7$ cells per mL. The initial density of the coliform could be determined from the growth of coliform in lauryl sulfate tryptose (LST) and by the detection of gaseous $CO_2$. LST was utilized for the incubation of *E. coli*. The sensor monitored *E. coli* growth by frequency response curves obtained in nutritional broth (pH 7.0) at 37 °C. Larger frequency changes in $CO_2$ gas were generated during bacterial metabolism. As the *E. coli* increased, the gas created covered the electrodes,

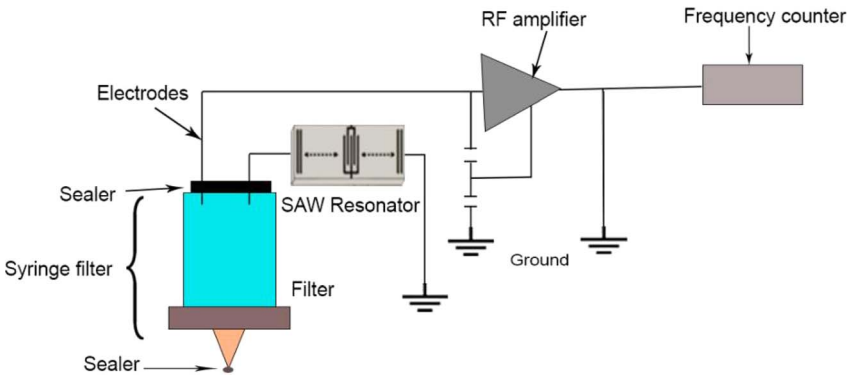


Fig. 7 Schematic of a commercial syringe filter.

producing a considerable fall in oscillator frequency and a rise in frequency as the *E. coli* multiplied.

This sensor system also allowed for real-time bacterial growth monitoring. In comparison with conventional bacterial plating methods, the operating procedure for this SAW sensor system is quite simple, and so it may be used and implemented by both scientists and non-scientists.

### 3.5 Ultrahigh-frequency SAW biosensors

This section describes a high-frequency SAW sensor for detecting *E. coli* bacteria. This type of acoustic biosensor has the potential to offer a simple, low-cost, and reliable method for detecting bacterial loads in complex liquids.[109] The sensitivity of SAW biosensors is determined by phase and frequency shifts, which are directly connected to wave types, the guiding layer size and material, and the sensing technology. SAW devices have been produced for high-frequency applications operating in the range of 100 MHz to a few GHz[110] and their high acoustic wave velocity is demonstrated by piezoelectric materials (Section 3.2), which allow producing higher-frequency SAW devices. These piezoelectric materials have a linear temperature–frequency relationship, indicating that as the temperature rises, the frequency also rises. A SAW device was reported using the piezoelectric substrate $LiNbO_3$, and it was found that as the center frequency rose to 2.55 GHz the insertion loss was reduced (13 dB).[110]

SAW-based high-frequency sensors have been researched by several authors. Thomas *et al.*,[111] Mujahid *et al.*,[112] Chen *et al.*,[34] Agostini *et al.*,[113] and Greco *et al.*[64] utilized high-frequency SAW sensors for chemical, biological, and biochemical applications. However, the majority of these studies were focused on chemical fields instead of pathogen detection. In 2007, Chang *et al.*[114] described (Fig. 8(b)) a new SAW (314.5 MHz) sensor for microbial count monitoring in biological cultures at ultrahigh frequencies. This sensor was used to count the number of bacteria in a biological culture. A pair of conductive electrodes were put into the transmitter's 314.5 MHz SAW stabilized oscillator. The frequency of the oscillator was changed when the electrodes were immersed in the culture solutions and the time was recorded. As the microbial metabolism changed, it produced a change in frequency (Fig. 8(a)); whereby the sensitivity increased in a parabolic manner with the resonance frequency of the device.[115] Finally, their detection limit was $\sim 10^2$ cells per mL in under 7 h, and the calibration curve of the detection times against the density of *E. coli* showed a linear correlation value (0.924). The authors also proposed that this sensor may be employed to transmit sensor signals wirelessly in dangerous areas, which would improve human safety aspects.

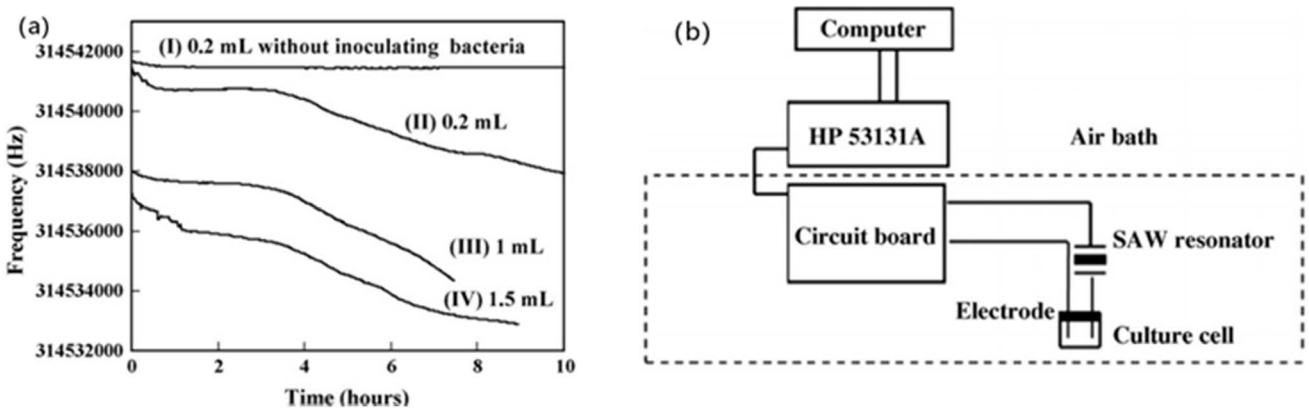


Fig. 8 (a) Effect of sample volume on the response of the SAW sensor. The sample was a nutrient broth (pH 7.0) inoculated with about 105 cells per mL *E. coli*. (b) New surface acoustic wave sensor for microbial count monitoring in biological cultures at ultrahigh frequencies.[114]

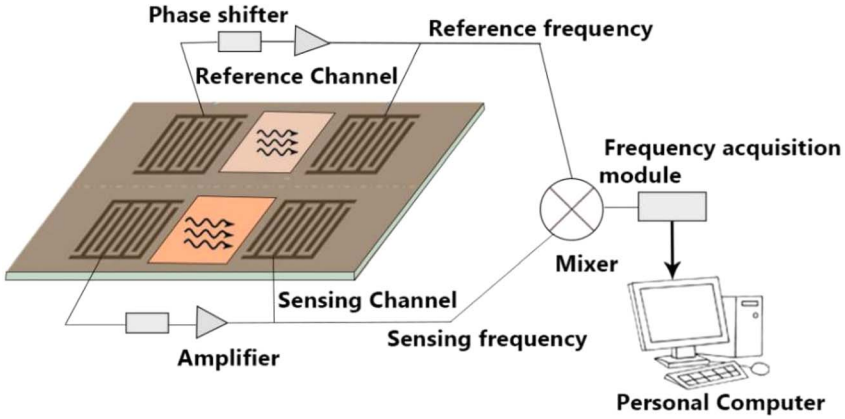


Fig. 9 Schematic of a dual-channel SAW sensor.

Based on the signal-to-noise ratio, the SAW sensor was proven to be more sensitive than the PQC sensor.[116] Even though the SAW sensor operates at an ultrahigh frequency, it demonstrated the same stability as a lower frequency PQC sensor. The SAW sensor also had higher sensitivity than the PQC sensor and a lower microbial detection threshold. If we compare it to traditional bacterial plating procedures, the process is quite simple and does not need the use of highly skilled experts.

### 3.6 Dual-channel SAW sensors

This section describes novel dual-channel SAW sensors that can detect *E. coli* bacteria in water. The fundamental construction of most dual-channel SAW sensors is similar. Fig. 9 presents an illustration of a dual-channel SAW structure with two delay lines; whereby one delay line works as the reference channel, while the other works as the testing channel. The testing channel performs all the mass adsorption procedures, whereas the reference channel gives a frequency reference. Because the two channels are formed of the same material, their reactions to changes in the environment are almost the same. As a result, the new frequency difference between the two delay line oscillators, $\Delta f$, may be considered the mass adsorption response, and the velocity changes of a SAW lead to corresponding oscillation frequency changes.[117] There is a basic frequency difference up to 1.3 MHz[118] between the sensing channel and the reference channel when a SAW oscillator system starts to oscillate.

Due to them offering some advantages, SAW-based dual-channel delay lines have been investigated by some researchers in various sectors in the past few years. These include Hur *et al.*,[117] Sakong *et al.*[119] and Fourati *et al.*[120] for DNA detection in liquids, Gray *et al.*[121] for digital diagnosis of HIV, and Wang *et al.*[118] for ultraviolet detection. In 2000, Howe and Harding[57] published their research based on a dual-channel surface acoustic wave device to identify two microorganisms of *Legionella* and *E. coli* bacteria in under 3 h followed by binding to a specific antibody. They used a Love-wave device in a dual-channel set-up, where one channel worked as a sensor and the other as a reference. Their research included a series of experiments that involved coating microorganisms on the sensor surface before adding the antibody. The antibody that particularly bonded to the SAW device was connected to the concentration of the bacteria adhered to its surface. A contactless SAW biosensor works by coating a layer of $SiO_2$ to protect the electrodes from the liquid. A digital frequency meter attached to a computer is used to measure the frequencies of both channels and the differential frequency is calculated to keep a record of the mass changes on the sensing channel. Additional research has been done to rule out the issues with using SAW devices in liquid phases. Typically, their detection limit is down to $\sim 10^6$ cells per milliliter in under 3 h.

Although, this sensor has the advantage of being able to measure in real time in systems with the delay lines operating at 200 MHz (ref. 119) and reduces the electronic measurement system complexity,[122] it has some limitations too. One of the biggest disadvantages is that dual-channel signals are also prone to crosstalk and the generation of noise.[123] We hope this sensor will be improved. Also, the molecular interactions on the transducer's surface should be more sensitive for higher oscillation frequency SAW sensors.

## 4. Summary

Many technologies for detecting foodborne pathogens have been developed in recent years to address food safety and public health issues, particularly with the growing demand for fresh and minimally processed foods. Table 5 shows the different types of detection methods for *E. coli* and their advantages using SAW sensors. Ultrahigh-frequency SAW sensors can be used for real-time bacteria monitoring in a biological culture. An important advantage of this sensor is its lower microbial count ($10^2$ cells per mL) and the ability to perform wireless measurements in hazardous environments. This technique is quite simple when compared to traditional bacterial plating procedures, and so does not need highly skilled personnel. Dual-channel SAW biosensors, SAW syringe filters, LGS-SAW, and $SiO_2$ nanostructure-based SAW sensors can also achieve bacteria monitoring in real time. These sensors work by grafting antigen–antibody on the surface. These methods are also

 

Table 5 Summary of SAW biosensors used to detect *Escherichia coli* O157 : H7

| | Detection range | Detection time | Temperature | pH | Waveforms | Principle | Advantages | Limitations | References |
|---|---|---|---|---|---|---|---|---|---|
| Ultrahigh-frequency SAW | $10^2$ cells per mL | <7 h | 30 °C | 6.5 | Rayleigh wave | Microbial metabolism affects loop capacitance inductance to change the resonant frequency | Simple, high sensitivity, does not require highly skilled technicians, low cost, and real-time bacteria monitoring | Time consuming, frequency dependent | 114 |
| Love-wave immunosensor | $10^6$ cells per mL | <1 h | 37 °C | 7.2 | Love wave | Antibody–antigen covalent binding, mass-loading effect leading to a resonant frequency shift | Ultrahigh stability, real-time monitoring, low cost, reusable, short detection time | High IL, guiding layer effect | 68 and 124 |
| Dual-channel SAW biosensor | $10^5$–$10^6$ cells per mL | <3 h | 25 °C | 7.4 | Love wave | Mass adsorption effect leading to a frequency difference ($\Delta f$) between the test channel and the reference channel | High sensitivity, good response, and short detection time | Influenced by temperature, pressure, competitive non-labeled methods | 57, 120 and 125 |
| LGS-SH-SAW | $\sim 10^6$ cells per mL | 2–5 h | 25 °C | 7.4 | Shear horizontal wave | Antigen–antibody specific binding changes cause a phase shift | Label-free detection, reduced attenuation, high-temperature stability and flexibility, good antibody bonding | Sensitivity, detection limit | 56 and 98 |
| SAW impedance sensor | $10^2$–$10^7$ cells per mL | 7 h | 37 °C | 7.0 | Rayleigh wave and Love wave | Changes in the impedance of the medium produced by bacterial metabolism cause changes in the frequency signal | Simple, real-time bacteria monitoring, can be used by both scientists and unprofessional | Exhibits high attenuation in liquids | 108 |
| SH-SAW biosensor | $6.54 \times 10^5$ CFU $mL^{-1}$ | 2 h | 30 °C | 7.3–7.5 | Shear horizontal wave | Antibody adsorption in the waveguide layer causes a resonant frequency shift | High specificity, repeatability, reproducibility, and lower detection time | Loss of energy, orientation of the crystal, corrosion | 11, 45 and 124 |

simple, low cost, and can easily detect bacteria in a short time. Another type of sensor called the Love-wave sensor can be used to identify biological species as well. Also, some research has focused on unique approaches incorporating multiple antibodies that allow complete *E. coli* bacteria to be immobilized on the sensor surface efficiently. The sensitivity of the Love-wave sensor is substantially higher than that of other piezoelectric sensors, such as the QCM. The capacity of the Love-wave sensor means it can identify the entire *E. coli* bacteria with excellent repeatability and specificity. This method maintains antibody/antigen interaction specificity and can give substantial results in less than 1 h with a detection limit of $10^6$ cells per mL. Love-wave devices are able to detect antibody concentrations as low as 1 ng $mL^{-1}$.[78] Among all the described methods, the Love-wave method is best for its fast detection and quick response time (<1 h).

## 5. Future perspective and conclusion

In this review article, we described various types of SAW-based methods for detecting *E. coli* bacteria and an overview of each sort of detection method is provided, as well as references to literature-based approaches. These methods are simple procedures and also easy to fabricate to detect a high amount of *E. coli* bacteria within a short time (<1 h). Several techniques for the fast identification of *E. coli* bacteria have recently been investigated and developed. However, most of them still need to be improved in terms of their sensitivity, selectivity, or accuracy before they can be used in any practical way. As a result, a reliable, accurate, fast, easy, sensitive, selective, and cost-effective detection technique is still desired.

In conclusion, fast and automated detection techniques for foodborne pathogens have a wide range of interesting applications. In the food sector and allied areas, such *E. coli* detection technologies would be a huge financial benefit. Even though *E. coli* continues to pose a serious hazard to human and animal welfare, numerous efforts are being undertaken to develop novel detection technologies. The benefits of SAW sensors are that they are extremely sensitive to changes in mass, density, and viscosity on the guiding layer and have huge potential and a significant possibility to see major advances that will enable them to become a vital player in the world of technology and point-of-care devices, especially given the ongoing desperate need for highly sensitive, selective, and cost-effective technologies for detecting microorganisms.

Though SAW devices represent a highly appealing technology for sensor applications, additional research is needed, particularly in the area of disease detection. These devices also have some challenges, including their detection limit, detection time, and specificity. Despite these challenges, SAW devices are still being investigated and exploited in sensing applications. Nanostructured SAW biosensors will be a key solution for future development to push the sensitivity and selectivity of SAW biosensors to new levels. Nanotechnology's extreme sensitivity sensing mechanism can enable nanosensing to overcome these challenges. In addition, the tendency of combining different approaches will lead to the development of new technologies or methodologies that will enhance the benefits of quick detection methods. Overall, these methods will become increasingly important in the identification and surveillance of foodborne pathogens in the future.

## Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

## Author contributions

Yujia Zeng: investigation and writing. Rui Yuan: visualization. Hao Fu: conceptualization. Zhangliang Xu: supervision and writing. Drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted. Song Wei: review and modification.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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