Recent advances of surface acoustic wave-based sensors for noninvasive cell analysis

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In the past years, the application of surface acoustic waves (SAWs) as sensors for biological applications has reached high relevance in the field of biotechnology. From rapid advances in designs and materials, new opportunities have emerged, especially for sensing of living cells. Additionally, the combination of SAW sensors with microfluidics and optical microscopy has expanded the market of possible applications. Differentiation of infected and healthy red blood cells or aggressive and nonaggressive tumor cells, and monitoring of wound healing, bacteria, or viral antigen concentrations via SAW-based sensors are only a few examples of recent achievements in cell biology. The rapid growth of this field requires frequent reviewing of the recent progress to maintain high research standards and promote future developments.

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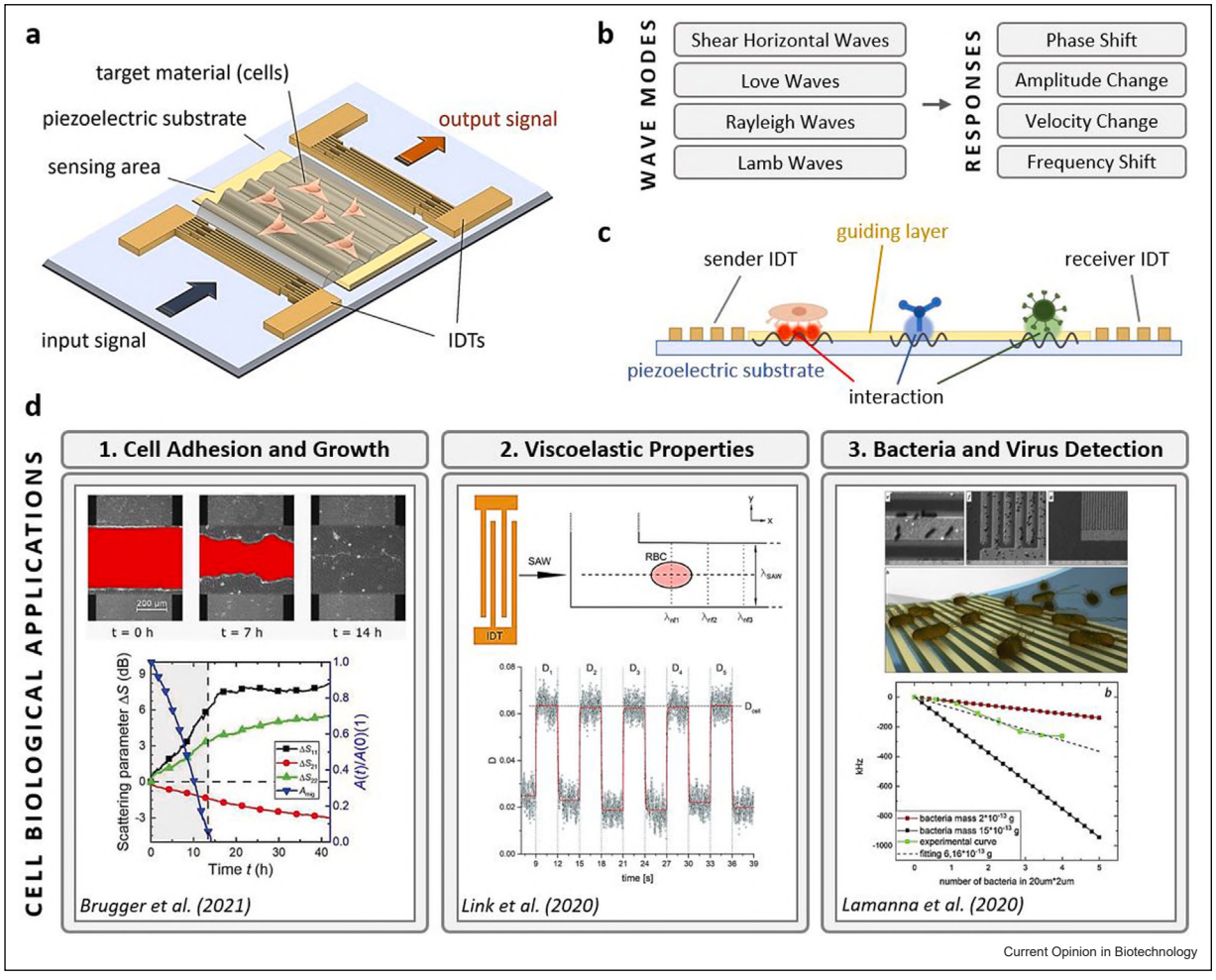
Introduction

Surface acoustic wave (SAW)-based sensors have become a widely used method for monitoring cells and cell mechanics. SAW as a noninvasive sensing technique have not only proven to be versatile, fast, and cost-efficient [1], but also allow for in-depth analysis of detected materials and their mechanical properties [2]. At the beginning of the 21st century, proteins were immobilized rapidly, reproducibly, and with high specificity on a SAW sensor [3]. Since cell membranes contain a large variety and number of proteins, it is challenging to transfer the high specificity of protein detection to cell detection. Nevertheless, these early studies paved the way for more detailed investigations on membrane proteins and their surface interactions: in 2008, *Saitakis* et al. used Love waves to identify the number of HLA-A2 molecules on LG2 and K562 cells on the sensor surface [4]. As one of the earliest studies employing SAW to detect viscous properties of biomaterials, *Laenge* et al. defined the measurable viscosity range of different protein solutions [5]. Since then, SAWs were also used to measure elasticity changes and localize diseased regions in whole-chicken breast tissue [6]. The SAW sensor technique has been proven to detect the adhesion process of fibroblasts [7], bacteria [8], and viruses such as bacteriophages [9], Coxsackie virus, and Sin Nombre virus B4 (SNV) [10]. To keep track of the rapid advances in SAW biosensing techniques and the widening field of applications, some reviews have been published: in 2012, *Saitakis* et al. discussed advances in acoustic biosensors for cell applications, and concluded that the technique should become more user-friendly and automized and that the damping effects of coatings could be a limitation [2]. The choice of smart coating materials, difficulties such as attenuating effects in liquids, and the maintenance of cell culture conditions on sensors were also highlighted in the 2019 SAW roadmap article [11]. As a promising future direction, an integration of actuator and sensor devices on one platform to simultaneously assemble and characterize biomaterials was proposed [11,12]. Reviews usually provide a wide overview of acoustic biosensing [2,13], so that the application of SAW sensors specifically to the field of cell biology has not been the main focus so far. To fill this gap, we here provide a compact review with special focus on SAW-based detection of cells and cellular properties. As a basis for understanding the sensing technique, we will first give an overview of the working principle and most frequently used SAW types. Then, we review the advances of SAW sensing over the past years, with emphasis on developments from 2019 to 2022, dividing the field of application into the three main categories: 1) cell adhesion and growth, 2) viscoelastic properties, and 3) bacteria and virus detection.

Figure 1



Summary of concepts for SAW sensing of cells. **(a)** Simple schematic of a SAW sensor for cell sensing in the delay line configuration. The output signal gives information on the concentration and properties of the target material deposited along the delay line (here: cells). Surface functionalization along the delay line is often employed to increase specificity, and, depending on the substrate and the generated wave modes, additional guiding layers may be required. **(b)** Overview of the most frequently used wave modes, the working principle, and measurable sensor responses. **(c)** Cross section of a SAW sensor with different biological substances on top of the guiding layer. **(d)** Overview of cell biological applications with selected examples from the past three years. The applications are divided into the three categories, and exemplary figures from sources of outstanding interest to each category are displayed: 1. sensing of cell adhesion and cell growth [28], 2. quantification of viscoelastic properties of cells [42], and 3. detection of bacteria and viruses [46]. Copyrights on figures: 1. ©Elsevier 2021, 2. ©Elsevier 2020, and 3. ©The Royal Society of Chemistry 2020.

Working principles of surface acoustic wave sensing

The working principle of SAW-based sensors, as illustrated in Figure 1, is based on the generation of an acoustic wave on the surface of a piezoelectric substrate. Interdigital electrodes (interdigital transducers, IDT) with an applied alternating current are fabricated on the substrate, typically a crystal, to generate regions of local tension and compression between the fingers of the transducer. This results in a mechanical wave propagating along the surface of the substrate [14]. The propagation of the wave at the surface underneath a sample toward a second IDT, produces a transmission output signal. Such a combination of sender and receiver IDTs forms a delay line. Mass loading results in changes of the output signal regarding the amplitude, phase, frequency, or velocity of the wave [15]. A detailed description of IDT types used in SAW sensors for live-cell studies is provided by *Mazalan* et al. [16].

In literature, four main SAW modes are reported for cell sensing: Love waves, horizontal shear waves, Lamb waves, and Rayleigh waves. Because of their high sensitivity and compatibility with fluids, Love waves are one of the most established wave modes for sensor devices in combination with living cells [17,18]. These are basically high-frequency shear-horizontal (SH) SAWs with a guiding layer that concentrates the acoustic

energy of the wave to the surface. Common guiding layers for Love waves are ZnO, SiO_2, PMMA (Polymethyl methacrylate), and SU-8 photoresist. *Xu* et al. reviewed the use of different guiding layers and their nanostructures in more detail [19]. Rayleigh waves, on the other hand, are rarely used for biosensing because they are highly attenuative with fluid load on the surface [20]. Lamb waves are generated in layered structures between two parallel surfaces, are highly dispersive, and consist of antisymmetric and symmetric wave types [21]. Fluid loading produces a leaky Lamb wave in the liquid, whose velocity depends on the product of the substrate thickness and the applied frequency [22]. Lamb wave frequencies are usually lower than the frequencies of Rayleigh waves, but artificial thin films such as ZnO or aluminum nitride (AIN) can be used to enable higher-frequency measurements [23]. SH waves are very sensitive to mass and viscoelastic loading because this results in a measurable frequency shift of the wave and makes them suitable for biosensing applications [24]. The amplitudes do not decay as fast as Rayleigh waves. Moreover, their sensitivity can be enhanced by using dielectric materials such as SiO_2 or polymers such as PMMA as thin guiding layers [25]. The advantage of SAW sensors over other acoustic sensor techniques, such as QCM (quartz crystal microbalance), is that SAW devices can easily operate at higher frequencies, and the sensor sensitivity rises with the square root of the frequency [2]. A more detailed summary of properties of the wave modes and materials used for SAW sensors is, for example, given by *Mandal* et al. [23].

Recent advances in surface acoustic wave-based sensing of cells

We here divide the research field of sensing cells and cell mechanics using SAW devices into three main categories, as illustrated in Figure 1: 1) cell adhesion and cell growth, 2) viscous and elastic properties of cells, and 3) the detection of bacteria and viruses.

In the first category, functions such as cell attachment and detachment, membrane interactions, proliferation, and migration on surfaces are monitored. Changes in the phase, frequency, velocity, or amplitude (insertion loss, *IL*) of the SAW correlate with the mass loading and concentration of adhered substances. The second category enables mechanotyping of properties such as stiffness, compressibility, deformability, viscoelasticity, and contraction forces of both adherent cells and cells in suspension. In this case, the change in propagation velocity, the phase change or the IL can be used to differentiate between cells or cell layers of different viscoelastic properties on the sensor surface. A combination of SAWs and acoustic streaming as actuators and an optical microscope as detector can be exploited to measure the properties of nonadherent cells. In the third category, the concentration of bacteria and viruses or the number of specific antigens on their membranes is determined. The mass loading and the resulting amplitude or phase changes provide information on the concentration of the adhered substance. While collagen or fibronectin are widely used coating materials for cell adhesion studies, the binding of specific bacteria and virus antigens often requires complex immobilization techniques and corresponding antibody coatings of the substrate. In the following, we will discuss recent advances in these three categories in more detail. A summary and annotations to all papers reviewed can be found in Table 1.

Cell adhesion and cell growth

For cell density and growth analysis, SH waves have become an established method: *Wang* et al. presented an SH-SAW sensor with ZnO guiding layer and a nanofiber scaffold coating as potential device for real-time measurements in 3D environments for bioassays or tumor studies. They monitored cell density of A549 cells and RAW264.7 macrophages by measuring the frequency shift [26]. The high stability and sensitivity of SH-SAW with Parylene-C as guiding layer and collagen coating has also been shown by monitoring the adhesion process of tendon stem cells at different concentrations [27]. Very recently, *Brugger* et al. showed the potential of SH-SAW devices as cell growth observation method, for example, in standardized wound-healing assays (Figure 1). They correlated the amplitude and phase change with the cell coverage of the sensor area during wound closure and were also able to quantitatively measure cell detachment processes [28].

The variety and specificity of coating materials, especially for Love wave sensors, has experienced an uplift during the past years. Chang et al. coated $LiTaO_3$ with assembled aptamers to detect MCF-7 cells with an overexpression of MUC1 protein on their surfaces, reaching a detection limit as low as 32 cells per ml [29]. In 2022, *Bonhomme* et al. proposed a novel SU-8 micropillar coating for Love wave devices that leads to sharp attenuations in the transmission frequency spectrum due to the excitation of resonance modes. These modes significantly enhanced the detection sensitivity of the Love wave sensor toward mass loading of polystyrene microbeads (220.3 m^2/kg) and temperature (7.96 kHz/°C) [30]. Presenting a technique for signal amplification, *Wang* et al. embedded gold nanoparticles on a commercially available SAW sensor to detect exosomes from HepG2 cells [31]. Another Love waves sensor differentiated HepG2 cell attachments with different Okadaic acid concentrations [32].

Combined devices for cell sensing employing SAWs, microfluidics, and optical microscopy have also been developed: *Sivanantha* et al. exploited acoustic

Table 1

A summary of studies on SAW sensors and their applications, divided into the categories: 1) cell adhesion and cell growth, 2) viscoelastic properties, and 3) detection of bacteria and viruses. Detailed annotations for the articles of special* or outstanding interest are given in the corresponding references.**

Studies	Setup information	Operating frequency	Detection targets	Measured responses	Results and annotations
1. Cell adhesion and cell growth					
Barié and Rapp (2001) [3]	36° rotated XY-cut $LiTaO_3$, horizontal polarized shear waves in combination with continuous flow system Coating: Parylene-C, carboxymethylated (CM) dextran, embedded antibodies	380 MHz	Bovine serum albumin (BSA), glucose oxidase (GOD), mouse Ig	Frequency shift, mass, or concentration of protein	Immobilization of biomolecules with high specificity on 3D matrix that binds up to 16 ng/mm^2 protein Sensitivities (4 Hz/ng for T-BSA, 59 Hz/ng for T-GOD) increased by two orders of magnitude compared with 2D layer
Laenge et al. (2003) [5]	36° YX $LiTaO_3$, horizontal polarized shear waves in combination with flow injection analysis system Coating: Parylene-C, OptoDex (dextran) as coupling agent, embedded antibodies	433.9 MHz	BSA, aqueous ethylene glycol solutions, and urease	Frequency shift versus mass/concentration of protein, viscosity	Reduction of flow cell volume to 4.8 µl for standard applications and 60 nl for extremely low sample consumption Measurements reproducible for viscosity range of 0.94–3.75 mPas; 3D matrix minimizes unspecific interactions from BSA adsorption
Tamarin et al. (2003) [9]	Love waves on ST-cut quartz Guiding layer: SiO_2 with AM13 monoclonal antibodies	~ 90 MHz	M13 bacteriophages	Frequency shift, concentration	Real-time graft of M13 bacteriophage immobilization Analytical method for phase velocity calculation and gravimetric sensitivity for complete multilayer structure
Saitakis et al. (2008) [4]	Love waves on Y-cut quartz substrate in combination with flow-through cell Coating: PMMA waveguide layer and 20-nm gold layer on top, protein G layer with bound monoclonal antibodies	110 MHz	HLA-A2/antibody complexes of Epstein–Barr virus-transformed human B-lymphoblastoid LG2 and chronic myelogenous leukemic K562 cells	Amplitude change (dB) versus number of cell-membrane receptor molecules on the surface	Novel sensing mechanism for investigating membrane interactions: 3D kinetic analysis enables measurement of detailed 2D kinetics and association and dissociation rate constants. Fast and label-free screening of membrane molecules and cell-membrane interactions in immune system
Guhr et al. (2008) [7]	SH waves on 36° rot YX $LiTaO_3$	85 MHz	L-929 murine fibroblasts	Change of resonance frequency, cell density	Increase of resonance frequency during adhesion process, rising slope with cell density Suitability of SAW devices for monitoring cell adhesion processes
Sivanantha et al. (2014) [33]	Concentrically focused IDTs on 128° rotated Y-cut X $LiNbO_3$ producing acoustic streaming in combination with optical microscopy	132 MHz	RBC	Deadhesion of cells under shear stress, applied power over time	Differentiation between malaria-infected, glutaraldehyde-treated, and healthy RBCs within 30 s at applied power of 500 mW for clinical diagnosis
Chang et al. (2014) [29]	Leaky SAW on $LiTaO_3$ with assembled aptamer	100 MHz	MCF-7	Mass loading, phase shift, and concentration	Capture of cells due to interaction between aptamer and overexpression of MUC1 protein on cell surface Detection limit: 32 cells/ml
Wu et al. (2015) [27]	SH-SAW on 36° YX $LiTaO_3$ with electrodes in dual-delay-line configuration Guiding layer: Parylene-C Coating: collagen type 1	131 MHz	Tendon stem cells	Acoustic loss (dB) and phase change (°) over time during adhesion process of cells at different concentrations	High sensitivity and stability for monitoring of the adhesion process of tendon stem cell (TSCs) at different concentrations Changes in phase and loss considered to be related to integrin–ECM protein interactions

Table 1 (*continued*)

Studies	Setup information	Operating frequency	Detection targets	Measured responses	Results and annotations
Wang et al. (2015) [26]	SH SAW on 36° Y-cut $LiTaO_3$ Coating: ZnO, 3D nanofiber scaffold	14.05 MHz	A549 cells, RAW264.7 macrophages	Mass loading, frequency shift, and cell density	Monitoring and cell density quantification of cells in suspension and cultured on 3D nanofiber scaffold Cell viability unaffected Potential tool for noninvasive longitudinal studies of 3D tumor cultures and real-time measurement in 3D environment for bioassays
Zhang et al. (2016) [32]	Love waves on ST-cut quartz	160 MHz	HepG2 cells	Cell attachment over time, Okadaic acid concentration	Potential tool for real-time convenient OA screening (wide linear detection range of various acid concentrations) Response of cell attachment related to initial cell seeding densities
Wang et al. (2020) [31]	Gold nanoparticles on SAW chip from SAW Instruments GmbH Guiding layer: SiO_2	Not specified	Exosomes from HepG2 cells	Phase shift	Signal amplification by AuNP Potential tool for early cancer diagnosis, detection of low-abundance exosomes in patient blood samples
Brugger et al. (2021)** [28]	SH waves on $LiTaO_3$ 36° XY-cut Guiding layer: 150-nm SiO_2	207 MHz	MDCK-II cells	Amplitude and phase change over time, mass loading, and area coverage	Observation method for cell growth and cell detachment processes and analyzation of standardized wound-healing assays Monitoring of culture conditions such as temperature and osmolality Acoustoelectric interaction as the main reason for phase shift in frequency- and time-dependent studies
Bonhomme et al. (2022)* [30]	Love waves on $LiNbO_3$ Y+36° Guiding layer: epoxy SU-8 with lattice of SU-8 cylindrical micropillars on top	35 MHz	Droplets, polystyrene microbeads, and sugar concentrations	Temperature, droplet size, sugar and mass concentration, and transmission signal (dB)	Sharp attenuations in frequency spectrum due to interaction of Love wave with resonances of pillars Linear frequency shift with mass of polystyrene microbeads
2. Viscoelastic properties of cells					
Li et al. (2012) [6]	Single-element piezoelectric ceramic with metal rod (shaker), detection of SAW with PhS-OCT system as pressure sensor (phase-sensitive optical coherence tomography)	~ 10 kHz	Different model phantoms made of chicken breast tissue	Distance to shaker, SAW amplitude/signal strength	Measurement of elasticity changes in both vertical and lateral directions to localize diseased tissue such as skin lesions Sensing depth ~5 mm, information on thickness, and width of inclusions
Xie et al. (2016) [43]	Piezo transducer generating acoustic waves in microfluidic chamber on gold-coated glass slide, in combination with optical (fluorescence) microscopy	100 kHz	HeLa, HEK 293, and human umbilical vein endothelial cells (HUVEC)	Shear force, deformability of different cells (diameter, aspect ratio)	Deformation measurements of cells by oscillating bubbles actuated by acoustic streaming enable differentiation between the different cell lines and cytochalasin D-treated and untreated HeLa cells. Combination of fluorescent and mechanical biomarkers

Table 1 (*continued*)

Studies	Setup information	Operating frequency	Detection targets	Measured responses	Results and annotations
Senveli et al. (2016) [35]	ST-cut quartz (SiO_2) excited in the x direction for Rayleigh wave generation in combination with microcavities and microprobes made of SU-8	196.7 MHz	Circulating tumor cells (MCF-7, MDA-MB-231, SKBR3, and JJ012)	Phase shift, elastic modulus	Distinguishing between single metastatic and nonmetastatic cell lines possible; high-frequency stiffness as possible biomarker for aggressiveness of cell Analyzation of sample volumes of < 10 ul using microcavities
Wu et al. (2019) [41]	Standing SAWs on $LiNbO_3$ in combination with PDMS microfluidic channel and optical microscopy	12.8 MHz	Polystyrene microbeads, A549, and HASM and MCF-7 cells	Phase shift, displacement over time, compressibility, and cell size	Cell trajectory impacted by compressibility and size Differentiation of compressibility and sizes of treated and untreated cell types and microbeads under different treatments
Wu et al. (2019) [36]	Love waves on 36° YX-$LiNbO_3$ guiding layer: Parylene-C	128 MHz	Tendon stem cells	Propagation velocity, propagation loss, viscoelastic cell layer thickness, and storage and loss shear modulus	Monitoring of TSC adhesion process Characteristic changes in propagation loss and velocity induced by viscoelastic cell layers with different loss or storage shear moduli
Devendran et al. (2019) [34]	Standing SAWs on 128° Y-cut X $LiNbO_3$ in combination with microchannel and optical (fluorescence) microscopy	48.5 MHz	MSC, MG63, L-929, and HaCaT cells	Cell attachment, viability, proliferation, stiffness, and metabolic activity	Cell-type-specific responses to acoustic stimulation at 400 and 800 mV Potential correlation between acoustic exposure, cell stiffness, and metabolism
Salari et al. (2020) [22]	Lamb waves generated by PZT disk, cover glass substrates of different thicknesses d with microfluidic channels Coating: fibronectin	$f\,d = 0.5$–10 MHz	MDA-MB-231 cells	Streaming velocity, flow pattern, and cell stiffness	Streaming flow pattern and magnitude influenced by cell morphology and acoustic actuation parameters Velocity of cell-induced microstreaming regulated by cell stiffness
Link et al. (2020)** [42]	Traveling SAW on $LiNbO_3$ in combination with microfluidic channel and optical microscopy	162.2 MHz	RBC	Static deformation, relaxation times	Simultaneous determination of elastic and viscous parameters of single erythrocytes Potential automatic principle for high-throughput blood screening
Wei et al. (2020) [37]	SH Love waves on piezoelectric quartz guiding layer: 3-μm SiO_2 film	160 MHz	HL-1 cardiomyocytes	IL, phase position, contraction, and viscoelasticity	Decrease in contraction force and stiffness by VRP-blocked calcium influx *In vitro* cardiac contractility evaluation
Zhang et al. (2021)* [38]	Love waves on ST-cut piezoelectric quartz Guiding layer: 3-μm SiO_2 and 50-nm Au layer	160 MHz	HL-1 cardiomyocytes	IL over time, cell density, and doxorubicin hydrochloride (ADM) doses	Decrease in viscoelasticity with increasing ADM dose Sensor as potential tool for early assessment of drug cardiotoxicity
Sarry et al. (2022) [39]	Love waves on Quartz AT-X+90 and $LiNbO_3$ 36Y–X Guiding layer: ZnO	11 433 MHz and 96.25 MHz	Viscous fluid	IL, phase shift, and fluid viscosity	Sensitivity to viscous fluids: ZnO/quartz more sensitive to viscosity, ZnO/$LiNbO_3$ more sensitive to mass effect Insertion losses and phase shifts due to viscous coupling, comparison between experimental and theoretical results

Table 1 (*continued*)

Studies	Setup information	Operating frequency	Detection targets	Measured responses	Results and annotations
Chavez et al. (2022) [40]	Love waves on LiNbO3 36Y–X in combination with fluidic chamber Guiding layer: 8-μm SU-8	30 MHz	SaOs-2 cells, viscous liquids	IL, viscosity, and phase change	Viscosity sensitivity: 0.89–3.3 cP (beyond focal adhesion zone) High penetration depth in viscous liquids as model for cell monolayers because of low frequency (sensitivity loss is overcome by thick guiding layer)
3. Bacteria and virus detection					
Berkenpas et al. (2006) [8]	SH SAW on langasite substrate Coating: Au and Cr layer, rabbit polyclonal IgG antibodies	92 MHz	*E. coli*	Phase shift over time	30:1 preference of *E. coli* to bind to anti-O157:H7 layer than to trinitrophenyl hapten layer 14° phase response for *E. coli* detection
Bisoffi et al. (2008) [10]	SH waves on 36° Y-cut X-propagating $LiTaO_3$ Guiding layer: SiO_2 with antibody coating	325 MHz	Coxsackie virus B4, SNV	Phase differential shift, virus concentration	Linear detection of increasing concentrations of viral particles within seconds Higher sensitivity for SNV, detection of SNV in complex solutions without analyte preprocessing
Jiang et al. (2014) [44]	Love waves on 41° YX-$LiNbO_3$ Guiding layer: SiO_2 with silane and antibodies	120 MHz	HA H1N1 antigen of influenza-A cells	Transmission, phase change over time, and concentrations of antigen	Detection limit of antigen: 1 ng/ml Immobilization of influenza-A hemagglutinin antibodies by triethodysilylbutyladehyde (ALTES) and triethoxysilylundecanal ethylene glycol acetal (ACTES) on surface
Yao et al. (2019) [45]	CM-dextran hydrogel chip and gold-layered sensor chips (from SAW Instruments GmbH) Coating: protein G and pAb antibodies	*(no information found)*	*E. coli* L-ASNase	Phase shift over time, concentration of L-ASNase	BS^3-activated amide coupling via protein G as immobilization method Potential pharmaceutical quality control method for L-ASNase medicines
Lamanna et al. (2020)** [46]	Lamb waves on AlN substrate fabricated on recyclable polyethylene naphtalate Coating: antibodies	R SAWs: 180 MHz L SAWs: 500 MHz	*E. coli*	Amplitude change, phase change, and mass loading	Lower detection threshold (5.54×10^5 CFU/ml, colony-forming units) on polymeric device than on silicon substrate Estimation of single *E. coli* mass using FEM simulations (9×10^{-13} g)
Tsougeni et al. (2020) [47]	Love waves on ST quartz on a LoC platform Guiding layer: S1813 photoresist with antibody coating	155 MHz	*S. typhimurium* in milk, *B. cereus*, Listeria, and *E. coli*	Amplitude, cell concentration	Integrated LoC platform for bacteria capture, lysis, and isothermal (LAMP) amplification Cost-effective and time-efficient protocol for analysis of DNA for food safety
Zhang et al. (2022) [49]	Love waves on piezoelectric quartz Guiding layer: SiO_2 with Au layer and antibody coating	152.5 MHz	Bacterial pneumonia biomarkers in EBCs	IL and phase, concentration	Proposal of an automated, high-throughput detection system for rapid detection of bacterial pneumonia biomarkers in EBCs Good linearity for immunobinding of C-reactive protein
Agostini et al. (2022)* [50]	Rayleigh waves on 128° YX-$LiNbO_3$ in combination with a microfluidic channel and antibody coating	740 MHz	Measles virions in human saliva	Frequency shift, concentration	Proof of concept for a SAW-based lab on a chip for detection of measles virions in human saliva down to 209 U/ml Combination of a low-frequency SAW actuator for sample recirculation and ultra-high-frequency SAW sensor array

streaming on $LiNbO_3$ chips and investigated the deadhesion of red blood cells (RBC) under shear stress. For applied powers of 500 mW, they were able to differentiate between malaria-infected, glutaraldehyde-treated, and healthy RBC within 30 s [33]. *Devendran* et al. used standing SAWs in a microchannel in combination with fluorescence microscopy to determine the possible effects of acoustic exposure on the functions of different cell types. The authors revealed a potential correlation between acoustic exposure at distinct power levels, cell stiffness, and metabolism, but only the adhesion of mesenchymal stem cells (MSC) was inhibited at both 400 mV and 800 mV due to their high mechanosensitivity. The functions of all other cell types were only affected at the highest power level [34]. Although this study did not explore the limits of applicable power densities in more detail, it underlines the importance that SAW sensors work in suitable operation ranges.

Viscous and elastic properties of cells

In the past years, Love waves on quartz or $LiNbO_3$ substrates were frequently used to monitor different cellular viscoelastic properties. *Senveli* et al. developed a quartz-based sensor in combination with microcavities in an SU-8 guiding layer to measure the high-frequency stiffness of small cell sample volumes, and were able to distinguish between single metastatic and nonmetastatic circulating tumor cells [35]. *Wu* et al. were able to detect tendon stem cell layers of different loss and storage moduli using a Parylene-C guiding layer on $LiNbO_3$ [36]. Another Love sensor with an SiO_2 guiding layer detected decreases in the contraction force and stiffness of HL-1 cardiomyocytes by VRP-blocked calcium influx [37]. A similar setup using ST-cut quartz was employed by *Zhang* et al. to determine drug cardiotoxicity: the authors showed that the viscoelasticity of the cardiomyocytes decreases with increasing doses of doxorubicin hydrochloride (ADM) [38]. A comparison of the performances of a quartz-based and a $LiNbO_3$-based SAW sensor device with ZnO guiding layers revealed that $LiNbO_3$ was more suitable for mass load detection, while quartz was more sensitive to viscosity changes [39]. Very recently, *Chavez* et al. combined Love waves on SU-8-coated $LiNbO_3$ with a fluidic chamber at a low frequency of 30 MHz. They showed that SAW sensors can detect mechanical changes beyond the focal adhesion points in cell monolayers with high penetration depth and sensitivity [40]. This verifies that quartz as well as $LiNbO_3$ combined with suitable guiding layers or microfluidic setups are both eligible setups to monitor viscoelastic properties of cells.

Various combinations of SAW devices with other detection methods have been optimized to widen the field of applications. For example, *Wu* et al. employed standing SAWs and optical trajectory tracking in a microfluidic channel to differentiate between cell types by their compressibility [41]. *Link* et al. induced static cellular deformations with traveling SAW on $LiNbO_3$ and were able to determine elastic and viscous parameters of single erythrocytes by optical observation of the cells' relaxation times (Figure 1) [42]. Moreover, acoustic streaming has proven to be useful for the actuation of bubbles [43] and inducing microstreaming around cells [22], hereby yielding information on cell deformability and stiffness.

Bacteria and virus detection

Similar as in cell adhesion studies, the sensing of bacteria and viruses usually exploits changes of phase and amplitude due to mass loading. Nevertheless, studies in this category usually require antibody coatings instead of common coatings on top of the respective guiding layers. These functionalized surfaces enable the observation of binding events of specific antigens on the bacteria's or viruses' shell to the sensor surface. *Jiang* et al. developed a Love wave-based device for virus detection on a silane and antibody-coated SiO_2 guiding layer with a detection limit of influenza-A antigen (HA H1N1) of 1 ng/ml. The researchers optimized the immobilization of HA antibodies on the sensor surface through effective chemical reagents and proposed this technique as potential tool for rapid clinical diagnosis [44]. *Yao* et al. successfully employed BS3-activated amide coupling via protein G to immobilize polyclonal antibodies on the sensor surface and verified their method by detection of different *E. coli* L-asparaginase concentrations [45]. In the context of contamination detection, *Lamanna* et al. used Lamb waves on an antibody-coated AlN substrate and showed that their polymeric device, fabricated on recyclable polyethylene naphtalate, yields a lower limit of bacteria detection than a silicon-based substrate (Figure 1) [46]. Via FEM simulations, they were able to estimate the mass of single *E. coli* cells, corroborating the potential of SAW-based sensors for flexible contamination control. Similar approaches for bacteria detection in the past two years were conducted by *Tsougeni* et al. and *Zhang* et al., who analyzed milk contamination [47,48] and bacterial biomarkers in exhaled breath condensates (EBCs) [49] using Love waves. Very recently, *Agostini* et al. presented proof-of-concept experiments for a SAW-based lab-on-a-chip (LOC) platform [50], where the researchers combined a SAW actuator for sample recirculation with a SAW sensor array for the detection of measles virions in human saliva. The limits of detection (LoD) in SAW sensor devices vary strongly for bacteria and viruses and depend on the employed materials and setup. While *Lamanna* et al. achieved a detection limit of 5.54×10^5 CFU/ml (colony-forming units) of *E. coli* on their polymeric device [46], *Agostini* et al. specified a significant limit of detection down to 209 U/ml of measles virions in their LoC setup [50]. The LoD for other biosensing techniques in contamination control, such as PCR or ELISA, are typically between 10^3 and

10^4 CFU/ml for bacteria [51]. Detection times of the other techniques are typically in the range of one to several hours [51], while advantages of SAW sensors are their real-time response and high automation potential.

Summary and conclusion

Over the course of the 21st century, the variety of applications for SAW sensors in the field of cell biology has significantly increased. Advances in the past 10 years have optimized the sensitivity and specificity of SAW sensors for biological applications down to a single-cell level, making it possible to determine the aggressiveness of tumor cells, differentiate between infected and healthy RBC, measure antigen concentrations on a virus' surface, monitor wound healing and bacteria contamination, and evaluate cardiomyocyte contractility. Highly efficient novel coating materials have been developed, and combinations of SAW as actuators with the field of microfluidics and optical microscopy now open new possibilities for sensing cellular properties, such as stiffness and deformability. However, the increased variety and versatility of SAW sensors come with challenges. For their highly specific detection targets, researchers usually develop customized designs, guiding layers and coatings. The large variety of devices and parameters often lacks sufficient description and uniformity, which can affect the comparability and transparency of studies. Standard sensor fabrication guidelines should be developed to obtain comparative and reproducible results. Sufficient information of a sensor's performance should be provided in consistent units, such as the sensitivity in units of cells per ml or CFU per ml. At the same time, it is important to keep investing in the development of novel coating materials and new concepts, such as SAW sensors integrated in LOC platforms, to achieve higher automation in the analyzation process. Nevertheless, the field of SAW cell sensing constantly reaches higher acceptance and applications among researchers, showing that SAWs are on their way to become a next standard sensing method in biotechnology.

Conflict of interest statement

We here confirm that the authors have no conflict of interest to declare.

Data Availability

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References and recommended reading

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