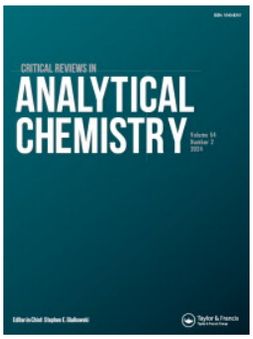

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Real-Time Biosensing Bacteria and Virus with Quartz Crystal Microbalance: Recent Advances, Opportunities, and Challenges

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ABSTRACT

Continuous monitoring of pathogens finds applications in environmental, medical, and food industry settings. Quartz crystal microbalance (QCM) is one of the promising methods for real-time detection of bacteria and viruses. QCM is a technology that utilizes piezoelectric principles to measure mass and is commonly used in detecting the mass of chemicals adhering to a surface. Due to its high sensitivity and rapid detection times, QCM biosensors have attracted considerable attention as a potential method for detecting infections early and tracking the course of diseases, making it a promising tool for global public health professionals in the fight against infectious diseases. This review first provides an overview of the QCM biosensing method, including its principle of operation, various recognition elements used in biosensor creation, and its limitations and then summarizes notable examples of QCM biosensors for pathogens, focusing on microfluidic magnetic separation techniques as a promising tool in the pretreatment of samples. The review explores the use of QCM sensors in detecting pathogens in various samples, such as food, wastewater, and biological samples. The review also discusses the use of magnetic nanoparticles for sample preparation in QCM biosensors and their integration into microfluidic devices for automated detection of pathogens and highlights the importance of accurate and sensitive detection methods for early diagnosis of infections and the need for point-of-care approaches to simplify and reduce the cost of operation.

KEYWORDS

Biosensors; magnetic nanoparticles; microfluidics; pathogens; QCM

Introduction

The quartz crystal microbalance (QCM) utilizes the piezoelectric principles to provide a simple, inexpensive, and precise way of measuring mass. The QCM is commonly utilized as a sensor for detecting the mass of chemicals adhering to a surface.[1] Scientists in sensor research have taken note of the technology for measuring molecular interactions due to low cost, ease of use, and high-resolution sensing QCM technology has a potential of detecting surface mass changes at pg/cm^2 levels.[1] Real-time readout data may provide quantitative information on analyte concentration, specificities of analyte-receptor interactions, affinities, and kinetics. Thus, QCM is also utilized for continuous, real-time analyte monitoring. With its capability of sensitive solution-surface interface measurement capabilities, QCM-based approaches have been developed for applications in predominantly analytical chemistry. The approach provides a wide detection spectrum. With low concentration samples, a monolayer surface coverage by minute molecules or polymer films may be identified. Its maximum detection range encompasses much larger surface-bound masses. QCM is a piezoelectric device in that a high frequency voltage is supplied to a piezoelectric crystal to cause oscillation, and its resonance frequency is monitored in real time as measured by frequency shift.

Infectious diseases are a continuous source of concern for global public health professionals and economic experts. Because pathogens contribute to the global spread of diseases such as food poisoning, sepsis, pneumonia, and tuberculosis, the threat posed by pathogenic bacteria to world health is serious.[2] Especially for bacteria and viruses, a prompt and accurate diagnosis is necessary to break the transmission cycle and prevent the development of illness. Traditional device-based diagnostic methods are time-consuming to employ at the point of care, making them ineffective for the optimum diagnosis of infectious infections. If hospitals had access to sensitive, quick, and affordable diagnostic tools that provided enough information for a treatment, they may have an advantage against infectious diseases. QCM devices have developed into a strong biosensing platform due to their capacity to detect and quantify a wide range of biomolecules without the need of a label. With their high sensitivity and rapid detection times,

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biosensors based on QCM have attracted considerable attention as a potential method for detecting infections early and tracking the course of diseases. There are other ultrasensitive methods of interest in this context like chemiluminescence-based and photoelectrochemical biosensors for cancer biomarkers.[3,4] A point-of-care photoelectrochemical detection by CRISPR-Cas12a is another alternative method as demonstrated for human papillomavirus-16 (HPV-16).[5] There are smartphone integrated versions of photoelectrochemical detection for cancer biomarkers.[6] Another promising method is photothermal-Pyroelectric Biosensor.[7] Smartphone-based electrochemical immunoassay was designed for SARS-CoV-2 nucleocapsid protein.[8] Hydrogel-based for visual detection and paper-based biosensors were reported for carcinoembryonic antigen.[9,10]

In this review, examples of QCM biosensor work will be summarized to encompass the wide variety of applications in infectious diseases and the recent approaches used in sample preparation, focusing on microfluidic magnetic separation techniques. A comprehensive account of the limitations and possible drawbacks of the QCM methods was not included in the scope of this work since excellent reviews on the subject are available.[11–16] QCM methods have been explained in detail for viruses and bacteria.[17–23] This review differs from them by highlighting the potential of microfluidic nanoparticles separation techniques in QCM sensing. Relatively new and improved concepts for QCM biosensors were explained with selected examples.

QCM biosensing method

The QCM chip is a vibrating disk constructed of quartz crystal with metal electrodes on both sides. Its vibration is caused by an electric field. The key idea behind this strategy is to determine by what percentage the mass moves when the frequency of the crystal resonator is changed. In the QCM transducer, a frequency voltage is supplied to the sputtered electrodes on both sides of the crystal, resulting in the creation of vibration. The fundamental frequency of a crystal is typically between 5 and 30 MHz, however this figure might vary depending on the crystal's thickness.[24]

The mass-per-unit-area variation and the resonator's frequency may be calculated using the QCM approach. Even a little amount of mass added or subtracted from the resonator's outer surface causes an instantaneous shift in resonance. The QCM may operate in the gas phase, liquid phase, or vacuum. This opens the way for the creation of sensor-based measurement analyzers capable of analyzing a wide range of sample types. This is critical for determining the deposition rate in thin-film production systems. It excels at identifying molecular interactions in fluids that lead to molecule adhesion on functionalized surfaces. Larger entities, like as entire cells and polymers, are also studied. QCM has also been used to explore biomolecular interactions, which has resulted in better disease diagnosis. Precise frequency readings can be acquired due to the capacity of measuring densities as low as 1 g/cm^2 by QCM. In addition to the frequency, the dissipation factor, which is comparable to the resonance bandwidth, is often evaluated. The dissipation factor is linked to the sample's viscoelastic properties and is used to compute the system's damping.

For the development of a QCM biosensor, the suitable recognition element must be very sensitive and specific to the intended target analyte. It must reliably detect and capture the target analyte, provide a rapid reaction, and exhibit excellent performance. In biosensor creation, recognition components such as nucleic acids, antibodies, cells, enzymes, and templated polymers have been utilized often. Since it is not essential to identify which molecules are linked for the label-free surface-sensitive method, QCM is a great technique for observing how molecules interact with one another in liquid samples. This is because their actual presence on the recognition surface produces signals. Recognizing viruses as quickly as possible is the single most important thing that can be done in public health applications to stop the spread of infectious diseases and get patients to the hospital as soon as possible. Biosensors based on QCM can fit neatly into this strategy for monitoring viruses. Recent pandemic of SARS-CoV2 has demonstrated that QCM applications may fulfill the demand for real-time monitoring, particularly of environmental samples. However, significant obstacles restrict the broad implementation of QCM biosensors. The sensitivity of the QCM device, which generates noise signals, is one of the greatest challenges. The adsorption of virus particles to surfaces with different attachment geometries, including direct adsorption to the surface, attachment through numerous anchors, and attachment *via* a single anchor, was correlated with the quantity of dissipation as a function of surface coverage rather than the internal properties of the particles. These investigations demonstrate that in many situations of biomolecular adsorption, the dissipation is not significantly impacted by the adsorbate itself, but rather by the affinity by which it is attached to the substrate.[25] As an observable variation from a linear dependence of QCM resonant frequency on mass deposition as predicted by the Sauerbrey relation, the sensitivity of QCM to the viscoelasticity of the adsorbed biomaterial may be established empirically and may limit its use in biosensing. A complete theoretical investigation was published to explain the deviation from the optimal mass response of soft overlayers upon liquid contact.[26]

QCM-based biosensors

Many targets, including bacteria and viruses, have been studied in relation to QCM biosensor signal. Additionally, bacterial biofilms may be investigated using QCM. Many excellent reviews have been published recently on various aspects of QCM detection of bacteria and virus pathogens, focusing mainly on advantages such as real-time, labelles and sensitive sensing abilities of QCM.[19,20,27–30] Therefore, we summarize notable examples of QCM applications involving pathogens in this review (Table 1). On the other hand, a specific caution was raised against bacteria detection by QCM by Olsen et al.[31] According to the traditional mass detection mechanism by QCM, a negative frequency

Table 1. Examples of QCM based studies for bacteria and virus detection.

Target bacteria	Ligand	Method	Sample	LOD	Reference
E. coli, B. subtilis spores	MIP	QCM	PBS or culture medium	3.72 x10^5–1.6·10^8 CFU/mL	[27, 39]
B. cereus	MIP	QCM			
E. canis	DNA probe	QCM	PCR product	22 copy number	[43]
M. leprae		Epitope-imprinted nanoparticles-modified EQCM		0.161 nM	[45]
E. coli	Antibody	QCM immunosensor	Vermicompost tea, PBS	$10^{2,}$ 10^6 CFU mL^{-1}	[40,46–49]
P. aeruginosa	Antibody	Graphene oxide QCM-D		na	[50]
C. jejuni	Antibody	mNPs, QCM immunosensor	Chicken meat sample	20 to 30 CFU/mL	[51]
A. actinomycetemcomitans	Antibody	QCM immunosensor	PBS	800 CFU/ml	
A. hydrophila	Antibody	QCM immunosensor	Fish tissue extract	6×10^6 to 10^8 CFU	[52]
B. melitensis	Aptamer	Magnetic NPs, QCM	*	10^3 CFU/ml	[42]
S. typhimurium	Aptamer	QCM	PBS	10^3 CFU/mL	[53]
AIV H5N1	Aptamer	Hydrogel based QCM	TBE Buffer	0.4 HAU	[54]
E. coli	Aptamer	QCM	PBS	1.46×10^3 CFU/mL	[55]
D. desulfotomaculum	Vancomycin	Magnetic NPs, QCM			[41]
H3N2 canine influenza virus (CIV)	Antibody	QCM immunosensor	Diluted Saliva	2^2 HAU	[56]
L. innocua	DNA Aptamer	QCM	PBS	1.6×10^3 CFU/mL	[57]
Classical Swine fever Virus	MIP	QCM	PBS	1.7 μg/mL	[58]

shift is what should be seen if bacteria are found to have attached themselves to the surface of a QCM sensor. However, according to the coupled-oscillator hypothesis, bacteria that are immobilized on the surface of a QCM sensor have the potential to trigger positive frequency fluctuations of up to 1.9×10^{-6} Hz per bacterium. Additionally, it has been shown that adherent bacteria that produce extracellular polymeric substances (EPS) modify the frequency shift in a negative direction by 1.7×10^{-6} Hz per bacterium. This finding is consistent with the traditional concept of mass loading. The difference in frequency shifts between a *Staphylococcal* strain that generates EPS and one that doesn't, was 3×10^{-14} g of EPS per bacterium. This constitutes a negligible portion of the total mass of a bacterium. Therefore, even a small percentage of an adherent bacteria's mass in an adsorbed molecular mass may have a significant impact on how the QCM signal is interpreted. To appreciate the fluctuations in QCM frequency that occur during bacterial detection, one must be acquainted with both coupled-oscillator theory and normal mass detection theory. This is owing to the fact that the molecular adsorption of EPS occurs after the attachment of diverse bacterial strains to the surface. Consequently, the molecule adsorption of EPS may have several distinctive effects on the QCM signal. Such details about the correct interpretation of the QCM frequency shifts upon capture of bacteria cells or virus particles can contribute to more applications of biosensors.

QCM biosensors for small molecules

Since small molecules have small size (less than 1 kD in definition), the frequency change will be quite small compared to bacteria or viruses. The QCM method is sensitive enough to detect small molecules, however, ability to detect small molecules in real samples are challenging. Hence, signal amplification methods are required for their detection by QCM biosensors. Two examples will be explained for signal amplification by nanomaterial use and circular amplification.

With the use of QCM biosensors, several small molecules linked to bacterial diseases have been identified. Guha et al.

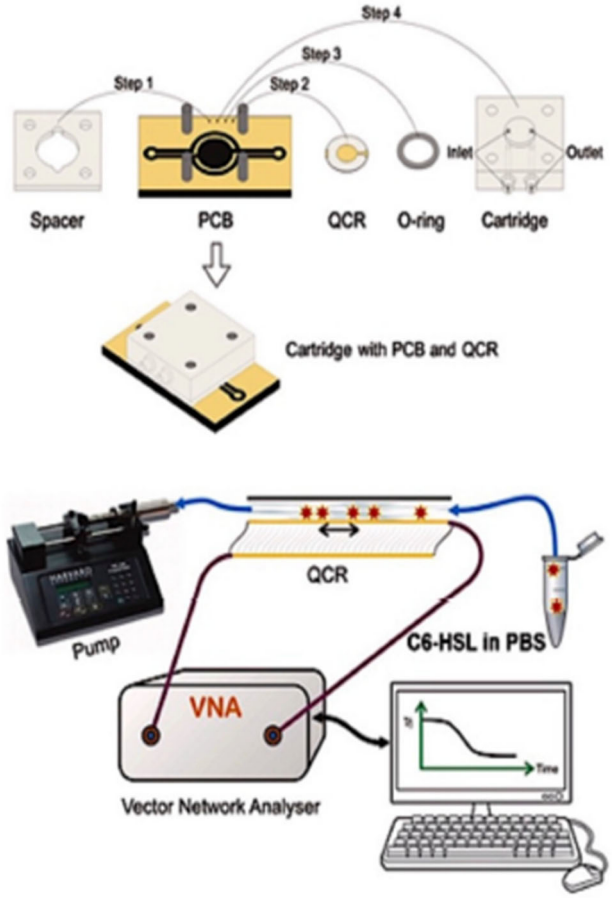

Figure 1. QCR experimental setup (Reprinted with permission from ref. [32] Copyright 2020 Elsevier).

created the N-hexanoyl-L-homoserine lactone (molecule participates in quorum sensing mechanism) biosensor employing a nano-molecularly imprinted polymer (MIP)-based quartz crystal resonator (QCR).[32] The ability to recognize a single molecule has significant industrial, environmental, and medical implications. Nano-MIPs were manufactured using the solid-phase molecular imprinting process. Figure 1 displays a QCR experiment. The sensor was built using a printed circuit board (PCB) that served as the electrical connection and a CNC-milled acrylic microfluidic cartridge that

allowed liquid to flow. Both parts were made of acrylic. The liquid sample is placed on top of the QCR sensor as part of the setup process (Figure 1). When all of the QCR sensors utilized in this experiment were combined into a single set, the liquid quality factor values totaled together. The sensor was controlled by a network analyzer, which enabled sweeps to be performed at both constant and variable frequency settings. The control interface for the recorder is a visual user interface that runs on a personal computer. The sample was given to the QCR sensor in a controlled and regulated manner using a syringe pump (Figure 1).

Similar to the above-mentioned quorum sensing molecule small molecules are difficult targets to detect since small mass will result in smaller frequency changes of QCM. Adenosine triphosphate (ATP) is the unit of energy currency for cells and biological processes. As a result, almost all cell types have ATP. In practically every kind of living cell, ATP may be detected. It governs the activity of cells as the source of energy for several metabolic processes. Additionally, it may be used to assess if a cell is still alive or whether it has been damaged. ATP detection may be used for hygiene monitoring in the food industry and for quick surface screening in hospitals.[33] In an attempt to increase the sensitivity of ATP detection, it was shown that ATP may be detected selectively and sensitively using an amplification method based on circular nucleic acid strand-displacement polymerization, aptamer recognition, and nanoparticle signal amplification.[34] These techniques were used to demonstrate that ATP could be detected at a LOD value of 1.3 nM. The identification of the ATP target starts with circular amplification technique, which includes an aptamer-based complex, two amplification reaction templates, and functioning AuNP probes. AuNP-functionalized probes act as a mass amplifier, hence improving QCM sensor signals. As a result of the DNA multiple amplification, the number of AuNP-functionalized probes, resulting an increase in the quality of the QCM signals has been increased. The examples can be expanded to include marker molecules for contamination, but for the purpose of elucidating the QCM capability, quorum sensing and ATP molecules are sufficient examples to address. The signal amplification methods of this kind using various nanomaterials have improved QCM applications for small molecules in recent years. In the next sections, examples with some of the nanomaterials like graphene oxide or hydrogels were presented in specific examples of QCM biosensors.

QCM biosensors for bacteria

QCM presents invaluable biosensing opportunities for monitoring pathogens in many examples. Food analysis, medical treatment, security surveillance, and environmental monitoring all need biosensors capable of detecting bacteria quickly, accurately, and sensitively. The spread of bacterial illnesses caused by infections has presented a danger to the general population's health. Numerous published research has shown the effectiveness of QCM in detecting microorganisms (Table 1). Many piezoelectric crystal-based pathogen detection technologies have been developed in recent decades. Conventional QCM biosensors generally include altering an Au chip surface with either an antibody that binds to the target bacterium or a cell-selective polymer screen to detect bacteria through molecular imprinting. There are alternative methods for food analysis like photoelectrochemical detection that can achieve similar goals.[35–38]

There are various strategies for preparing the surface of a target-specific chip sensor surface for subsequent QCM detection. For MIP, large targets, such as bacteria or viruses, are typically imprinted on the surfaces using sedimentation or stamping technologies (Figure 2a). In this method, the template is pressed into a pre-polymerized spin-coated layer and then removed after polymer hardening. MIP involves immobilizing the template on a functionalized surface using an appropriate linker. After that, a polymer layer of the specified thickness is produced *in situ*. In the final phase, the template bacteria (or virus) are eliminated, thereby making the corresponding cavities accessible for analyte recognition through specific binding. In a typical QCM-immunosensor strategy for *E. coli* O157, magnetic separation of target cells was first step by antibody capture. The purified sample was then applied on the QCM chip surface functionalized by antibodies (Figure 2b). The antibiotics can be used to detect bacteria using QCM. Sulfur releasing bacteria was determined by a method based on vancomycin functionalized magnetic beads. The purified bacteria cells were capture on the QCM surface covered by vancomycin (Figure 2c). The same strategy of QCM detection of purified bacteria can be achieved on aptamer functionalized chip surfaces (Figure 2d). QCM has been used for hybridization-based detection of bacteria genomic DNA (Figure 2e).

QCM biosensors have been reported as an improved solution to biosensing challenges for many interesting health problems. Due to its association with animal morbidity and death, *Ehrlichia canis*, a disease transmitted by arthropods and intracellular parasite bacteria, is gaining attention. *E. coli* O157:H7 is a gram-negative pathogen with rod morphology, and a sporeless bacterium that is representative of gut bacteria. *Bacillus subtilis* is a well-known model microbe for studying spor forming bacteria. A MIP surface was created on gold surfaces for QCM detection of spores of *Bacillus subtilis* and bacterial cells of *E. coli*.[27] Bulk molecular imprinting technique was used to build this QCM biosensor. It was feasible to identify bacteria or spores using QCMs with a two channels setup. In a separate study, Yilmaz et al. created whole-cell-imprinted QCM biosensors to identify *E. coli*.[44] Leprosy, also known as Hansen's disease, is moderately contagious and is caused by the *Mycobacterium leprae*. Kushwaha et al. utilized nanoparticles imprinted with epitopes to build an electrochemical QCM (EQCM) biosensor that could detect *M. leprae* bacteria at high sensitivity.[45]

QCM platform can be coupled with nanomaterials for improved bacterial detection. The antibacterial capabilities of graphene oxide (GO) are well recognized, but the kinetics of bacterial adhesion on GO surfaces under ecologically important circumstances are mostly unexplored. QCM with dissipation analysis (QCM-D) was utilized to investigate the

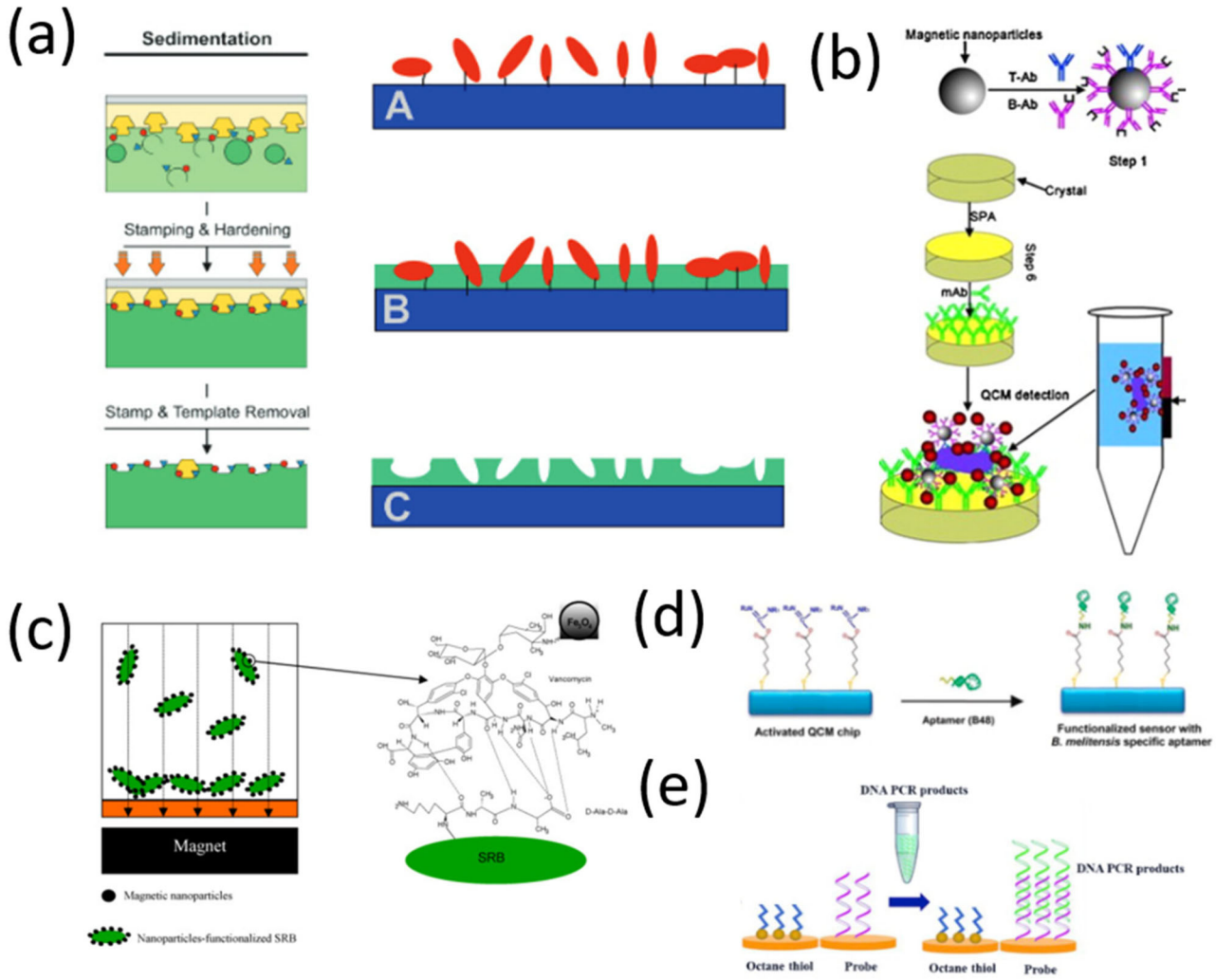

Figure 2. Bacteria detection strategies for QCM detection. (a) MIP, (b) Antibody, (c) Vancomycin, (d) Aptamer and (e) Hybridization based detection principles were presented. (Reprinted with permission from ref. [39] (a) Copyright 2016 Elsevier; ref. [40] (b) Copyright 2011 Elsevier; ref. [41] (c) Copyright 2010 Elsevier; ref. [42] (d) Copyright 2019 Elsevier; ref. (e)[43] Copyright 2019 Elsevier).

capture of a model organism, *Pseudomonas aeruginosa* on a GO surface under varying ionic strengths. Maximum bacterial adhesion at modest ionic strengths (200–400 mM) was obtained which is crucial for optimized sensor conditions.[35] Such studies are crucial for expressing the robustness of the QCM detection systems in different environmental conditions and in combination with different technologies. Numerous aptamer based QCM sensors have been documented, including a hydrogel based aptasensor for Avian Influenza Virus (AIV) H5N1.[13,17,59] Due to the QCM's sensitivity to ambient noise brought on by temperature fluctuations, the hydrogel-based QCM aptasensor proved unsuitable for use in the field. Nevertheless, microfluidic-based QCM systems may readily circumvent the issue of temperature stability by including a constant temperature device.

In order to demonstrate the robustness of the system, another QCM application for the detection of pathogenic bacteria can be seen in the food industry, where the analyte is examined in highly complex and processed matrices. In the food production industry, *E. coli* is one of the most dangerous foodborne infections. This bacterium has the capability of causing renal failure, anemia, bloody stools, and bloody diarrhea. Using antibodies against *E. coli* strains, several immunosensors have been produced. Biofunctional micro/nanobeads were employed as signal amplifiers to enhance the sensitivity of a QCM immunosensor for detecting *E. coli* O157:H7 in PBS buffer.[48] The municipal wastewater treatment plant's sewage sludge has a high quantity of microbiological pathogens. Pathogens might potentially infiltrate the human food chain using sewage sludge in agricultural regions without sanitary management. Using *E.coli* antibody covered QCM chip surface specific for a surface antigen of *E. coli* bacteria, 10^2 CFU/mL of *E. coli* O157: H7 was determined in vermicompost tea samples spiked with the pathogen.[46]

According to the simplest interpretation of the QCM measurement data, differences in resonance frequency are associated with variations in the mass of the sensor layers. However, according to Sauerbrey, this only applies to layers that retain their rigid structure in a vacuum. Since the detecting layer in biosensors is in direct contact with a liquid, viscoelasticity causes the acoustic wave to weaken as it approaches the liquid and passes over the detecting layer.

After the sensing layers have been exposed to bacteria, it is necessary to assess their viscoelastic properties to guarantee they have not been changed.

Inflammation, brought on by bacterial plaque, destroys the tissues that hold teeth in place, resulting in periodontal disease and eventual tooth loss. *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) are only two of the bacterial species that have been linked to advanced periodontal conditions. Quantitative evaluation of *A. actinomycetemcomitans*, a periodontal bacterium, was performed using a QCM) immunoassay with a detection limit of 800 CFU/mL and a dynamic range below 2.32 10^6 CFU/mL.[60] In fish farms, bacterial infections may result in substantial financial losses, necessitating the need for quick detection techniques. There is considerable interest in label-less biosensors which is quick in monitoring aquatic pathogens, but little research has been conducted on them so far. Using a QCM immunosensor device simplifies the detection of the fish and human pathogen *Aeromonas hydrophila* (*A. hydrophila)* in comparison to the usual indirect ELISA approach. After covalently cross-linking an anti-*A. hydrophila* antibody on gold surface of the sensor chip, a QCM immunosensor system was reported to be able to determine bacterial attachment within 5 min as a change in frequency with a high degree of specificity for bacterial loads between 6.25 and 100 g ($6x10^6$ to 10^8 CFU).[52] Samardzic et al. created a MIP surface coated on a QCM chip for *E. coli.*[61] This sensor is used to detect targets in biological matrices in the real samples in real time. To illustrate the viability of this approach, *E. coli* samples were examined utilizing paused flow. A LOD of 1.6 10^8 cells/mL value were obtained in short assay time. Schnettelker et al. took use of in-situ polymerization to build bacterial MIP layers directly on the surface of the transducer. Due to this, the transducer's sensitivity increased, resulting in a reduced detection limit.[39] This method is especially useful with coating planar surfaces, and it is compatible with sensitive microfluidic device transducers. QCM method was also used to study lectin binding properties to study bacteria–host interactions.[62]

QCM biosensors for viruses

The average size of a virus is less than 100 nm, which is a lot smaller compared to the average size of bacteria. QCM biosensors have been said to be able to find many viruses, just like they can find bacteria. Potential bioreceptors for virus detection as well as bacteria are biomacromolecules, mostly antibodies and aptamers, and molecularly imprinted polymers.[17]

The H3N2 canine influenza virus (CIV) is a virus with an eight-segmented, single-stranded, negative-sense genome and confined in an envelope. It belongs to the genus of Influenza A virus (IAV). QCM was used to find out that saliva samples from the field were infected with the H3N2 canine influenza virus (CIV) in 10 min.[56] Dogs with an H3N2 CIV infection may have fever, runny nose, loss of appetite, lack of energy, and mild to fatal respiratory symptoms.

As expressed for bacteria section, aptamer-QCM coupling is also a valid strategy for detecting viruses. Although virus particles have simpler structures (hence smaller size) compared to bacteria cells, QCM can be applied to virus detection. Aptamers have been chosen for several viruses, including severe acute respiratory syndrome (SARS), influenza, dengue virus, rabies, norovirus, human immunodeficiency virus (HIV), hepatitis B (HBV), and vaccinia virus. Polydopamine-based MIP approach with QCM detection was reported for Hepatitis B.[63] Numerous aptamers based QCM sensors have been documented. A QCM aptasensor based on hydrogel for monitoring Avian Influenza Virus (AIV) H5N1 was reported.[54] This research indicated that the aptamer-functionalized hydrogel may be utilized to detect viruses with enhanced sensitivity. However, the hydrogel-based QCM aptasensor was not suitable for usage in the field due to the QCM's susceptibility to ambient noise caused by temperature variations. Nevertheless, microfluidic-based QCM systems may readily circumvent the issue of temperature stability by including a constant temperature device.

All of the above examples of virus or bacteria detections targeted the presence of the bacterial cell, virus particles or their individual molecule antigens directly. Another approach for pathogen detection is the determination of serological biomarkers in the biological samples. A recent example is the design of a rapid detection device for Hepatitis B antigen.[63]

Magnetic nanoparticles and microfluidic systems in sample preparation

One of the major challenges with biological samples is the interference of matrix components on the frequency changes. Magnetic particles functionalized with affinity molecules have been used as pretreatment method before feeding into QCM systems in many examples. Recent examples of the use of magnetic particles in microfluidic designs for prepurification of bacteria or virus and their integration into QCM is notable in that an automated system can easily be obtained. Most bacterial solutions include only minimal amounts of bacteria in addition to a range of other components, pretreatment is necessary prior to sensing in order to separate and concentrate the bacteria of interest. For the diagnosis of microbial diseases and the body's response to an infection, numerous sample types are available, such as swabs, sputum, saliva, urine, stool, tissue, and blood-based specimens. Proper and standardized sample collection and preparation are essential for obtaining accurate and reproducible test results in this context.[64] Depending on the type of detection format, sample purification is usually required for obtaining stable detection signal. Liquid samples like blood can simply be diluted to reduce the signal interfering agents or they can be filtered out. Another common sample preparation for biosensors is removing interfering substances by differential centrifugation or precipitation. However, the

sample preparation is desired to be as simple as possible for on-site diagnosis applications. Handling steps, instruments required and their energy supplies, training for users are the main handicaps for real-life applications.[65] Magnetic beads based separation methods stand out from other sample preparation techniques by providing quick, easy, automation-prone and fitting in small devices.

Due to the extraordinary properties of iron oxide magnetic nanoparticles (MNP), including their high magnetic susceptibility, superparamagnetism, and biocompatibility, this pretreatment approach stands out from others.[66] In particular, the nanoparticles' strong magnetic susceptibility makes them a good choice for biological applications. When the bacteria or virus of interest are caught by MNPs with recognition molecule functionality, it is straightforward to separate and concentrate the bacterium-MNP complexes using an external magnetic field.[67] Enrichment using magnetic particles is an effective method for increasing the quantity of analytes in a sample while eliminating contaminants that interfere with QCM sensing. The most significant drawback of QCM devices is that when device size reduces, noise levels tend to increase. This is because increasing the ratio of surface area to volume makes the measurements less stable. Another drawback of QCM is that it is difficult to locate analytes in solution, and it is also impacted by the relative humidity of the air. Using magnetic nanoparticles, it is possible to gather bacteria or viruses of interest fast and efficiently. The procedure is usually very simple and takes less than 30 min in many reports. Magnetic beads or microfluidic magnetic systems were also used as capture agent in other biosensor formats like photoelectrochemical assays.[68–70]

A QCM was developed to reliably detect *Salmonella enterica* serovar typhimurium cells in milk samples.[71] Being an enteric pathogen infecting both humans and animals and widespread around the world makes this bacteria an important target for biosensing systems including QCM. Using a technique of magnetic microparticles separation, the LOD value of 100 CFU mL^{-1} was obtained with QCM detection. An aptamer-based magnetic separation technique, target pathogens, was enriched rapidly, and QCM analysis enabled precise and real-time monitoring. The aptamer-based QCM system consisted of magnetic beads that bind Salmonella and are captured by aptamers. This provides a method for separating Salmonella specifically. Because magnetic separation approach efficiently and rapidly captures the target bacterial cells, sending them to a QCM flow cell device in PBS buffer revealed that Salmonella could be accurately identified in less than 10 min with much less device noise. In another example of application of magnetic bead separation, Bayramoglu and colleagues reported that a nanoparticles-mediated approach had LOD of 10^3 CFU/mL, making them acceptable for detecting *Brucella melitensis* in milk.[42] Aptamer-immobilized magnetic nanoparticles were initially used to separate the desired target microorganisms from the sample matrix. The same aptamer sequence was then attached to a QCM chip to use for the detection of *B. melitensis* (Figure 3). To detect and differentiate *B. melitensis* cells in a variety of biological materials, a biocompatible polymer (poly(ethylene glycol)-methacrylate) was first grafted onto magnetic nanoparticles and was utilized to limit the chances of the ligand or other undesirable molecules interacting with the support. Following that, epoxy groups capable of directly binding biological molecules were created by grafting a reactive monomer onto the surface of magnetic nanoparticles. The QCM sensing surface was then loaded with the *B. melitensis*-recognizing aptamer. Target bacteria were adhered to the QCM chip surface using the eluent from the magnetic separation technique. Figure 3 covers an overall application of this approach on food samples which is a two-step procedure; separation and washing of matrix components by magnetic pull-down and QCM detection.

Using a QCM biosensor, *D. desulfotomaculum*, a marine pathogenic sulphate-reducing bacterium, was detected selectively using the conjugates of bacteria and magnetic nanoparticles attached on an Au chip sensor by application of an external magnetic field based on the increased response of magnetic nanoparticles functionalized with vancomycin.[41] The bacteria were incubated with vancomycin- magnetic nanoparticles for 30 min as part of the experiment. Linear range between 1.8 x 10^4 and 1.8 x 10^7 CFU/ml, a linear correlation between the QCM response and the logarithm of the bacterial concentration was observed. Other than the typical receptor-ligand recognition used in standard QCM, an external magnetic field was utilized to enhance and receive signals.

Microfluidic particle analysis technique is gaining significance in biosensing due to its size and accuracy. Its use in cell separation enables sample preparation for precise and sensitive biosensing. Particle separation in a microfluidic system may be achieved *via* a variety of approaches. Using acoustic sorting, particles suspended in a liquid may be managed. Due to the acoustic radiation force produced by the ultrasonic field when it generates a standing wave in the microchannel, particles move in the direction of the wave node or pressure. The three primary types of acoustic tweezers are standing wave tweezers, moving wave tweezers, and streaming acoustic tweezers. Acoustofluidics, the combination of acoustics with microfluidics, has the potential to transform the approach to cell manipulation. There are numerous applications for sound-trapped microbubbles in the field of microfluidics, including generating gradient and chemical waveforms, mixing and pumping fluids, manipulating particles, and controlling microflow. Using physical principles and immune-related factors, it was possible to distinguish circulating tumor cells from pure blood with acoustic techniques.[72]

Urinary tract infections are one of the most common health problems, and if not treated immediately, they may progress to severe complications such as sepsis. Uropathogenic *E. coli* accounts for about 75% of all urinary tract infections. Treatment of infections requires an early diagnosis, but the current methods are costly, time-consuming, and insensitive. Current point-of-care approaches are ineffective because they cannot identify the bacteria responsible for the sickness. Instead, they depend on indirect

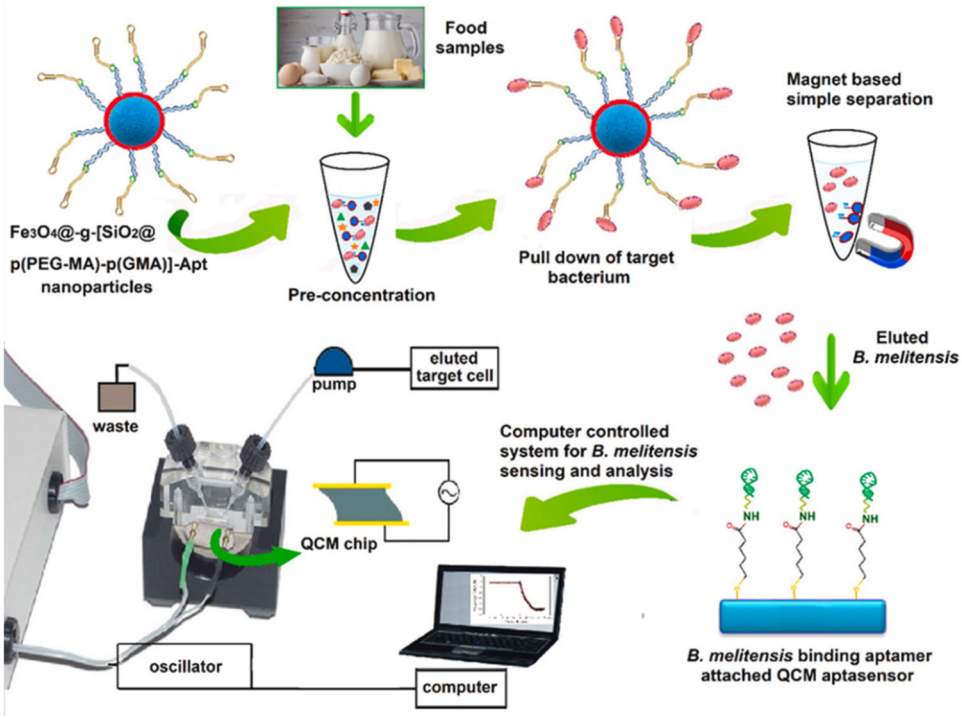

Figure 3. QCM aptasensor device and aptamer-based affinity magnetic separation approach (Reprinted with permission from ref. [42]. Copyright 2020 Elsevier).

indicators such as elevated urine nitrate levels. Call et al.[42] developed a magnetophoretic method for detecting *E. coli* in the urinary tract. The movement generated by a magnetic field is known as magnetophoresis. A magnetically labeled targets in an aqueous electrolyte solution comparable to the composition of biological fluids face away from the source of the magnetic field, not toward it. This, thus, opens the door to a very selective separation approach in which the magnetic field source strongly attracts the labeled targets due to the targeted binding of paramagnetic or ferromagnetic particles, allowing them to be easily separated from the rest of the matrix components. Magnetophoresis is an effective technique for removing target cells from complicated matrices in order to study them. To regulate flow in microfluidic devices using magnetophoresis, complex and expensive equipment is generally required. Microfluidic paper-based analytical devices (PADs) and magnetophore-sis may be utilized to regulate flow without the need of a pump, therefore simplifying and reducing the cost of operations. The detection limits of early magnetophoresis PADs were greater than what was required for clinical application, but they were still competitive with older methods. Magnetophoresis was reported with LOD value of 2.40 10^2 CFU/mL to identify *E. coli*. This was accomplished by pumping buffer to hybrid PADs with capillary action in hydrophilic channels.[73]

Magnetotactic bacteria (MTB) are capable of constructing and forming chains of magnetite through a biomineralization mechanism. This is an evolutionary adaptation for sensing direction by complex subcellular mechanisms to construct linear chains of magnetite nanocrystals.[74] Magnetosomes are strain-specific lipid membranes that contain magnetic nanoparticles of various sizes and shapes (around 100 nm, spherical or other irregular shapes). MTB-produced magnetosomes facilitate biomedical applications due to their homogeneity, narrow polydispersity indices, ferrimagnetic crystallography, high thermal stability, and aggregation resistance, as well as their lipid coatings, which facilitate surface chemistry and the creation of recombinant proteins that target to the magnetosome membrane.[75]

Microfluidic devices provide process automation and downsizing as advantages, which is proposed to be used in conjunction with magnetic pretreatment of pathogen microorganisms. Using procedures such as the reaction of carbodiimide hydrochloride and N-hydroxy succinimide as well as the silane treatment, the surface of MNPs may be easily functionalized.[76] The modified surfaces are subsequently coated with recognition molecules including polymers, aptamers, and antibodies.[42,77] If recognition molecule-functionalized mNPs are coupled to the bacteria, permanent magnets may be employed to safely separate complexes of bacteria and mNPs. This allows mNPs to function as recognition molecules. In contrast, standard separation approaches based on mNP require a variety of steps, such as binding, washing, and enrichment, that include mixing and transfer. When the quantity of bacteria in a sample rise, it may take between thirty minutes to several hours to identify the bacteria of interest. Microfluidic devices, which have the capacity to automate procedures, reduce cost, and reduce the size of the device, might be utilized to discover solutions to these problems.[78] Using microfluidics-based automation and downsizing methods, it is possible to develop devices that are portable and can be handled by anybody without the requirement for specific training.[79] There is a synergistic effect when MNPs and microfluidics are used in

conjunction. This action makes pretreatment and detection far more successful than with conventional methods.

More complex designs of microfluidic devices were constructed for suspending magnetic particles in liquid phase for efficient capture from large quantity samples and washing them. Low quantities of samples that are handled by microfluidic devices might be a drawback in dilute samples. The invention of MNP-based virtual filters may also be used to get over the constrains of microfluidics to process huge quantities of samples.[80] When a magnetic field was supplied to a glass tube containing a solution of antibody-functionalized mNPs, the particles were aligned in the same direction as the field. This resulted in wall of particles. When the flow force exceeded the magnetic force holding the mNPs together, a liquid was pumped into the microfluidic tube, causing the mNPs to migrate in the other direction of the liquid flow (Figure 4). In contrast, according to Lenz's law, injecting a liquid into a tube wrapped in copper tape would produce a magnetic field perpendicular to the direction in which the liquid is traveling. Therefore, the mNPs may stay contained inside the copper-tubes at 2.5 times the flow rate at which they could have been contained within the tube. *E. coli* O157:H7-added buffer samples were injected such microfluidic design to provide binding of the bacterial cells to the mNP wall like a virtual net. As measured by an ATP luminescence method, the detection limit for *E. coli* was 10^2 CFU/mL in a total assay time of 15 min, including 10 min for the isolation and separation steps.[80]

Using a passive microfluidic methodology that is desirable for lab-on-a-chip applications, a method for the purification of bacterial cells is created. In a spiral microchannel device, microfluidics for continuous multi-particle separation was shown. Because the microchannel is spiral-shaped, the forces of inertia and drag hold neutrally buoyant particles (or living cells) at equilibrium position close to the microchannel's inner wall. There is a significant association between the size and position of a particle or cell. Archimedean spiral microchannel device of 5 loops was deployed to show that four particles may be brought into focus while remaining distinct. This concept served as the device's foundation. Polystyrene particles with diameters of 7.32 nm, 10 nm, 15 nm, and 20 nm were chosen for this experiment because they are the same size as red blood cells. Comparable to the performance of conventional microfluidic separation techniques, the device was able to separate around 87% of the fluids on average. In another study, a simple Lab-on-chip application that can rapidly size-separate particles were reported based on a square microchannel in a straight line between two intake and two exit reservoirs. Dielectrophoresis (an electric field that is not uniformly distributed throughout the channel wall) was achieved by implanting asymmetric three-dimensional electrodes along the channel.[81,82] Distinct reservoirs on the chip gathered particles of varied sizes. A pressure differential between the input and output reservoirs causes the main movement for efficiently removing particles with diameters ranging from 5 to 10 mm, such as yeast cells, white blood cells, and latex.[83] Acoustophoresis can be another alternative technique for cell and particle separation.[84]

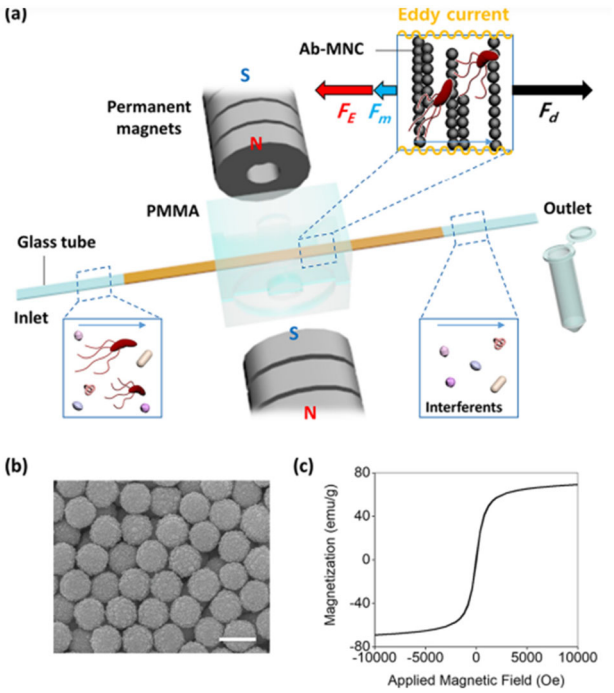

Figure 4. (a) Schematic of isolating pathogenic bacteria using antibody functionalized magnetic nanoparticles net in Cu-glass tube. Copper tape was around the tube (Yellow). (b) SEM image, (c) Magnetization curve of the particles (The figure was reproduced from ref. [80] by permission of ACS).

Conclusions

QCM biosensor research has demonstrated its enduring importance with proved effectiveness in the study of molecular interactions on solid surfaces in biological samples. However, main limitation of QCM technique originates mostly from the interference of complex biological sample components. MNPs may rapidly identify solutions to problems associated with the quality of QCM signals in complex matrices because they possess a sensitive signal transduction mechanism and a recognition event. They are also beneficial since they are inexpensive and simple to manufacture. Advantages include the capacity of MNPs to attach to analyte molecules, therefore enhancing their concentration at the QCM surface in a buffer of choice. MNPs have been to be used in microfluidic systems to separate microparticles owing to their persistent magnetic moment. These devices may be controlled by varying external magnetic fields and captured cell by ligands can be enriched in minutes from complex biological matrices. MNP-based separation and microfluidic-based QCM sensing can readily be merged onto the same chip. This would enable the integration of several analytical operations, such as sample pretreatment and QCM detection analysis, into a single device.

Authors' contributions

Conceptualization, supervision and writing—review and editing, V.C.O. and M.Y.A; writing—original draft preparation, F.B., M.K., S.U, B.C., A.D.D., G.B. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

The authors report there are no competing interests to declare.

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