Chapter 14

Biosensor Platforms for Rapid Detection of *E. coli* Bacteria

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Additional information is available at the end of the chapter

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Abstract

Risks of contamination with the well-known food pathogen *Escherichia coli* are increasing over the years. Therefore, rapid and portable technologies using different types of advanced devices named biosensors with various transduction capabilities (electrochemical, optical, or acoustic) were developed and seem to offer the most elegant solutions for research communities and final users-humans. Thus, integration of microfluidic biochips/biosensors into smartphones offer the real-time detection of any infection with *E. coli*, helping doctors in proceeding immediately with the clinical treatment. The present chapter will discuss about the analytical performances of biosensors and microfluidics such as selection of substrates, type of (bio)functionalization, low limit of detection, specificity, and response time for monitoring different *E. coli* strains. Thus, it is possible to rapidly identify (30–90 s) very low concentrations of *E. coli* (10^1 CFU/mL) down to a single bacterium in real samples (water, urine, milk, beef-meat) by simple integration of an angle scatter method and microfluidic-cellulosic pads (μPAD) loaded with micro-/nanoparticles functionalized with either polyclonal anti *E. coli* antibodies or with DNA strains into a portable device—a smartphone. Such biosensor configuration can also be used for the detection of other types of microorganisms with potential human and animal health concerns.

Keywords: biosensor platforms, electrode (bio)functionalization, *E. coli*, microfluidics, smartphone

1. Introduction

Coliforms or bacteria are commonly found in the digestive tracts of animals, humans, plants, and soils. Most coliforms do not cause diseases. However, the existences of "coliforms" with four genera (*Escherichia, Citrobacter, Enterobacter*, and *Klebsiella*) are indeed used as

indicators to monitor their potential enteric pathogen contamination of waters [1]. One of the hundred strains of the bacterium *Escherichia coli* named *E. coli* O157:H7 is known as one of the most dangerous (for instance, Europe 2011) [2] gram-negative emerging cause of many foodborne and waterborne illnesses [3], bloody diarrhea (hemorrhagic colitis) [4], hemolytic-uremic syndrome (HUS) causing the kidney or renal failure, and hemolytic anemia (loss of red blood cells), which may lead to death, especially in children [5]. *E. coli* microorganisms (width ~ 0.6 μm, length ~ 1.6 μm) [6] produce the Shiga toxin that causes inflammation and secretion of intestinal fluids. Antibiotics and anti diarrheal medicines (such as Imodium) are not recommended for treating *E. coli* O157:H7 pathogenic infections.

Over the years, beside the relatively time-consuming and expensive conventional detection methods (e.g., evaluation of microorganism morphology, counting the bacteria colonies with Violet Red Bile Agar [7] after 72 h, investigations through the polymerase chain reaction (PCR) for amplification of low contents of bacterial nucleic acids), several methodologies with rapid detection using either laboratory instrumental methods (e.g., chromatography, infrared/epi-fluorescence microscopy [8], bioluminescence, flow laser cytometry and immunomagnetic separation [9], fluorimetry [10], etc) or portable, sensitive, and specific devices named biosensors have been reported [11]. Thus, in 1989, Karp's group [12] reported the development of the first biosensor using stable-light-emitting *E. coli* cells. Technically, a biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is in direct spatial contact with a transducer element [13]. Today, on the ISI Web of Science are reported more than 1220 publications about different biosensor configurations for the specific and selective detection of *E. coli* bacterial cells.

Biosensors are classified either as bioaffinity sensors when the biological recognition entity is an antigen/antibody (named immunosensors) [14] or a single strain DNA or ssDNA/RNA sequences (named DNA-/RNA sensors) immobilized onto a solid support via a linker molecule [15] that interact specifically with a target (named the complementary ssDNA-sequence to the ssDNA-probe), enzyme sensors, receptor ligand-binding sensors [16], and whole cell biosensors (named microbial sensors); or in function of the type of used physical transducer in electrochemical, optical, piezoelectric, and calorimetric (bio) sensors.

On the other hand, since early 1997 [17] bioluminescent genetically engineered *E. coli* microorganisms were intensively used as sensitive and friendly bioreporter's tools for screening the genotoxicity and cytotoxicity of various classes of water pollutants, endocrine disruptor's compounds, explosives [17, 18], and nanoparticles [19–22].

This chapter presents the latest developments for the detection of *E. coli* strains using electrochemical, optical, and acoustic biosensors and their integration in microfluidic platforms as well as the next generation of portable biosensors using smartphones (**Figure 1**, **Table 1**).

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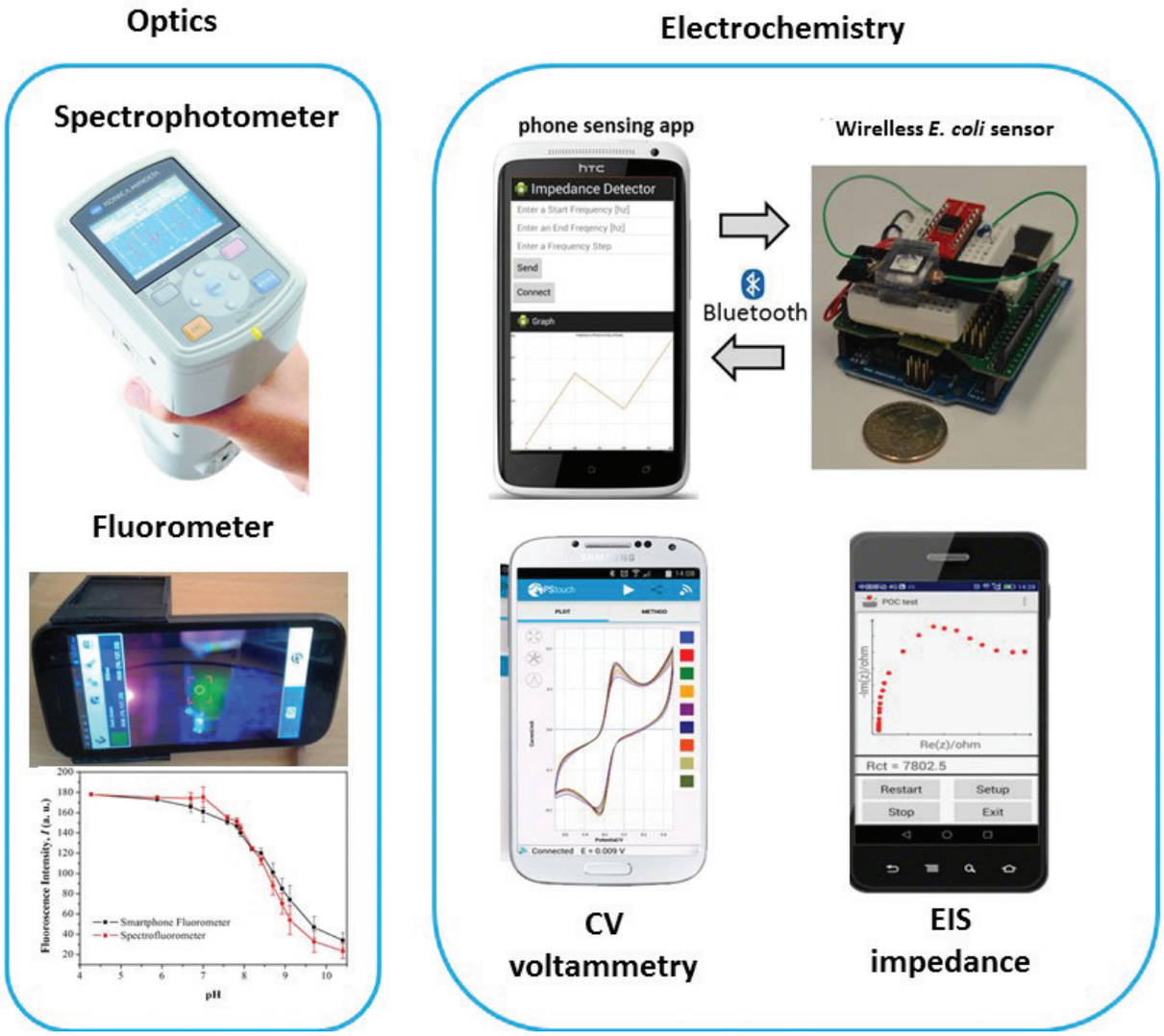

Figure 1. Optics and electrochemistry connected to smartphones for the detection of *E. coli* bacteria. For instance, an electrochemical system includes an Android cellphone (HTC ONE X), a Bluetooth shield (Seeed SLD63030P), a micro controller (Arduino), a chip for impedance converter network analyzer (AD 5933), and packaged sensor [66]. Also an Xcode (Apple, CA, USA), which allows users to take pictures at the four specific angles of scatter detection (15°, 30°, 45°, and 60°) at a fixed distance is reported [62]. CV—cyclic voltammetry, EIS—electrochemical impedance spectroscopy.

2. Biosensor platforms for the quantification of *E. coli*

Optical-based surface plasmon resonance and acoustic quartz crystal microbalance biosensors are known for their analytical performances in terms of label free and real-time detection capabilities, fast and stable response time, and target low detection limits. Furthermore, electrochemical impedance spectroscopy (EIS) label free biosensors are suitable for electrical characterization (such as double layer capacitance, solution resistance, electron transfer resistance, and Warburg impedance) of biocatalytic transformations on electrode surfaces after each biofunctionalization step [23].

Transduction method	Biofunctionalization	Strain of *E. coli* (analyte)	Media	LOD/range (CFU/mL)	Ref
Electrochemical					
	Polyclonal-antibodies 10 μg mL^{-1} for 1 h, RT/DTSSP for overnight, 4°C/AuSPEs *(Configuration 1)* Thiolated-polyclonal anti *E. coli* antibodies 200 μg mL^{-1} overnight, 4°C/AuSPEs *(Configuration 2)*	Wild-type *E. coli* (CECT 515)	PBS buffer + redox probe	3.3 ($5.0–1.0 \times 10^8$)	[24]
EIS	Biotinylated Con A-*E. coli*/Au-SPE	Wild type *E. coli* (CECT 515)	PBS buffer + redox probe	5.0×10^3 (5.0×10^3–5.0×10^7)	[25]
	Antibodies (24 h, 4°C)/indium-tin oxide (ITO)—interdigitated glass array microelectrode	*E. coli* O157:H7 (ATCC 43888)	PBS buffer + redox probe	1.0×10^6 (4.36×10^5–4.36×10^8)	[26]
	Antibodies (1 mg/ml, moisture chamber 24 h, 4°C)/GPTRES/ indium-tin oxide (ITO)—glass	*E. coli* O157: H7	PBSM buffer	6×10^5 cells/ml	[27]
	Monoclonal antibodies, 24 h, 4°C/ EDC-NHS/MPA-SAM/Au-glass *(Substrate preparation)* Bacteria + Biotinylated-*E. coli* polyclonal antibody/streptavidin superparamagnetic beads (*Fluid preparation*) (syringe pump, 20 min incubation time for immunocomplexes formation)	*E. coli* O157:H7 (strain B1409) (Prajna Biology Technique Shanghai)	PBS buffer	50 (50–500)	[56]
FET	0.1% Tween20/Ethanolamine/ Polyclonal Antibodies/ PBSE-linker/Graphene	*E. coli* K12 ER2925	PBS buffer + traces of LB medium	1.6×10^7	[40]
Optical					
SPR	UV-light, 20 min, RT/Bacteria + Mixture of (HEMA/EGDMA/ deionized water/MAH/the initiator AIBN)Dry bacteria/GA/ APTES/Glass-slide (20 min, flow measurements)	*E. coli* (Sigma-Aldrich)	Aqueous solutions	1.54×10^6 (0.5–4.0 McFarland*)	[52]

Transduction method	Biofunctionalization	Strain of *E. coli* (analyte)	Media	LOD/range (CFU/mL)	Ref
Reflectivity	*E. coli* suspensions or lysates, 1 h/K-7α12/OAK/MPTMS/ $PSiO_2$-wafer	*E. coli*, ATCC 8739	Saline solution	10^3 cells/mL (10^3–10^5 cells/mL)	[28]
Chrono-fluorimetry	ssDNA 25 mer of 0.1 μM + EB/ Ether wash/DMT-HEG, RT, 4 h/ GPTES under argon at 80°C, 24 h/ Silica optical fiber (10 min, flow measurements, response speed of 20–40 s 40°C)	*E. coli* K12	PBS solution	10 pmol cDNA	[1]
Prism	NH_2-ssDNA of 100 μM, 1 h at 37°C//HEPES, 10 min/PDC, 2 h/10 min at 100°C/APTES in water + ethanol, 20 min/ SiO_2-TiO_2—15 layers (air measurements after hybridization at 37°C for 2 h)	*E. coli* O157 : H7 of Gene ID: 957271	Air	8.13 μM DNA	[29]
FT-RIS	Antibodies 40 ng/mL, 24 h at 4°C/GA RT, 2.5 h/APTES 1.5 h/ NaOH/Oxidized pSi-wafer (flow measurements, 15 min, dark conditions)	*E. coli*	MHB solution	10^3 (10^3–10^7)	[30]
Acoustic					
	Antibodies/Protein A/SAM/ QCM-crystal	*E. coli* O157: H7		10^3–10^8 (CFU within 30–50 min)	[31]
	Iron magnetite-NPs coated with Streptavidin and polyclonal biotinylated antibodies/*E. coli* cells/Antibodies/ProteinA/QCM-crystal (7.99 MHz, NPs = 145 nm, 30 min stop-peristaltic flow measurements)	*E. coli* O157: H7	PBS buffer	10^8	[32]

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Transduction method	Biofunctionalization	Strain of *E. coli* (analyte)	Media	LOD/range (CFU/mL)	Ref
	Fe_3O_4—NPs coated BSA-Steptavidin/Biotinylated-DNA/BSA-blocker/ssDNA-thiol/QCM-crystal (8 MHz, NPs = 145 nm, flow measurements)	*E. coli* O157: H7	PBS buffer	2.67 x 10^{-2} and 10^{-12} M DNA	[33]
	Ethanolamine/Polyclonal Antibodies/MUA-SAM/QCM-D (peristaltic flow measurements)	*E. coli* MRE 162	Air + PBS	2.4×10^7	[34]
	Biotinylated polyclonal IgG antibodies/Avidin/NHS-PEG-biotin/Cysteamine/Au/Cr/LGS-crystal (syringe pump flow measurements at RT)	Fluorescent labeled *E. coli* O157:H7	PBS + BSA	~10^6 cells/mL	[35]
QCM	Mixture of twice heated *E. coli* (first at 60°C for 30 min and second at 37°C prior use) and solute lyophilized TAL/Ag-QCM-crystal (9 MHz, peristaltic flow measurements at 37°C)	*E. coli* in mixture with *TAL*	Culture medium	~10 cells/mL	[51]
	Imprinting the bacteria on overoxidazed polypyrrole film/QCM-AT-crystal	*E. coli* (Japan)	Sterilized water	10^3–10^9	[6]
	0.1% SDS Wash/Polymerization/Bacteria/Pre-Polymerization at 70°C, 15 min/DMMP-MIP (~300–400 nm) spin-coated onto QCM-sides with screen-printed dual electrode structures (10 MHz, peristaltic flow measurements at RT)	*E. coli* strains b and w (Sigma)	Aqueous solution	0.1 mg/mL (0.1–5 mg/mL)	[36]

Transduction method	Biofunctionalization	Strain of *E. coli* (analyte)	Media	LOD/range (CFU/mL)	Ref
	UV-light 20 min, RT/ Bacteria+Mixture of (HEMA/ EGDMA/deionized water/MAH/ the initiator AIBN)/Dry/Rinse-PEA/AM 12 h/QCM-crystal (7 min, flow measurements)	*E. coli* (Sigma-Aldrich)	Aqueous solutions	3.72×10^5 (0.5–3.0 McFarland*)	[52]

Legend: MPA-Mercaptopropionic acid MPA; GPTRES-(3-glycidoxypropyl)trimethoxysilane: PBSM-6.7 mM $[Fe(CN)6]^{3-/4-}$ (1:1 mixture), $MgCl_2$ and 3 mM 5-bromo-4-chloro-3-indolyl phosphate disodium salt hydrate; Con A-concavalin A; AuSPEs-gold screen printed electrodes; DTSSP-3,3′-dithiobis[sulfosuccinimidylpropionate]; PDC-diisothiocyanate; HEPES-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EB-ethidium bromide intercalator; DMT-HEG-dimethoxytrityl hexaethylene glycol; $PSiO_2$-oxidized porous silicon nanostructure; OAKs-oligomers of acylated lysines; K-7α12 - synthetic antimicrobial peptide with similar name K-$[C_{12}K]_7$, where K-lysine; MPTMS-mercaptopropyltrimethoxysilane; MES-morpholinoethanesulfonic acid; AEE-2-(2-aminoethoxy)ethanol; GPTES-3-Glycidoxy-propyldimethoxymethylsilane; CFU-Colony Forming Units; SAM-self-assembled monolayer; MUA, 11-mercaptoundecanoic acid, QCM-D, quartz crystal microbalance with dissipation shifts capability; BSA-bovine serum albumin; TAL-tachypleus amebocyte lysate; PBSE-1-pyrenebutanoic acid succinimidyl ester in dimethylformamide (DMF); LB-Luria Bertani medium; MIP-molecular imprinted polymer; DMMP-dimethyl methyl phosphonate; RT-room temperature; SDS-sodium dodecyl sulfate solution; AM-allyl mercaptan; PEA-pure ethyl alcohol; GA-glutaraldehyde; APTES-(3-Aminopropyl)-trimethoxysilane; HEMA-2-hydroxyethyl methacrylate; EGDMA-ethylene glycol dimethacrylate; AIBN-α,α′-azoisobutyronitrile; FT-RIS-Fourier Transformed Reflectometric Interference Spectroscopy; MHB-Mueller-Hinton broth; *MsFarland-McFarland Equivalence Standards [37]-units for adjusting densities of bacterial suspensions: 1.5–12 x 10^8 cells (SPR) and 1.5–3 x 10^8 cells (QCM)range (for Ref. [52]); PBS solution of pH 7.4 containing 10 mM $[Fe(CN)6]^{3-/4-}$ (1:1) for Interdigitated array (IDA) microelectrode (1.4 cm x 0.5 cm)-each electrode had 25 digital pairs with 15 μm digit width, 15 μm interdigit space, and a digit length of 2985 μm.

Table 1. Different biosensor platforms for detection of *E. coli* bacteria.

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2.1. Electrochemical biosensors

In January 2016, it has been reported for the first time an amperometric detection of PCR products (longer DNA chain's) by using three modified magneto working electrodes (m-GEC) with silica magnetic beads (0.05 mg) functionalized with either digoxigenin-tagged amplicon of the *eaeA* gene for *E. coli* (151 bp), fluorescein-tagged amplicon of the *invA gene for Salmonella enterica* (278 bp) or streptavidin-tagged amplicon of the *pfrA gene for Listeria monocytogenes* (217 bp) as specific carriers for independent magneto-genosensing amperometric investigations of single-tagged amplicons originated from three bacteria strains: *E. coli* K12, *S. enterica* Typhimurium LT2 and *L. monocytogenes* DSM20600 (DSMZ), respectively. Such amperometric magneto-silica beads platform was using HRP-labeled antibodies and the same redox mediator (HQ-hydroquinone) and substrate (H_2O_2) (**Figure 2**) in a unique electrochemical cell connected to a multichannel potentiostat and was able to identify 0.04, 0.13, and 0.05 ng/μL DNA of *S. enterica*, *L. monocytogenes,* and *E. coli,* respectively, in about 3 h, including PCR amplification time [38].

Label-free impedimetric immunosensors-based ITO substrates modified with epoxysilane and anti *E. coli* antibodies were used for detection of *E. coli* O157:H7 over a large linear working range (10–10^6 CPU/mL) with a limit of detection (LOD) of 1 CPU/mL were also reported. Moreover, the authors demonstrated the specific binding of *E. coli* O157:H7 to the antibody-patterned surface (only 20% of non specific bacteria) (**Figure 3**) using control bacteria strains such as *Salmonella typhimurium* and *E. coli K12* [39].

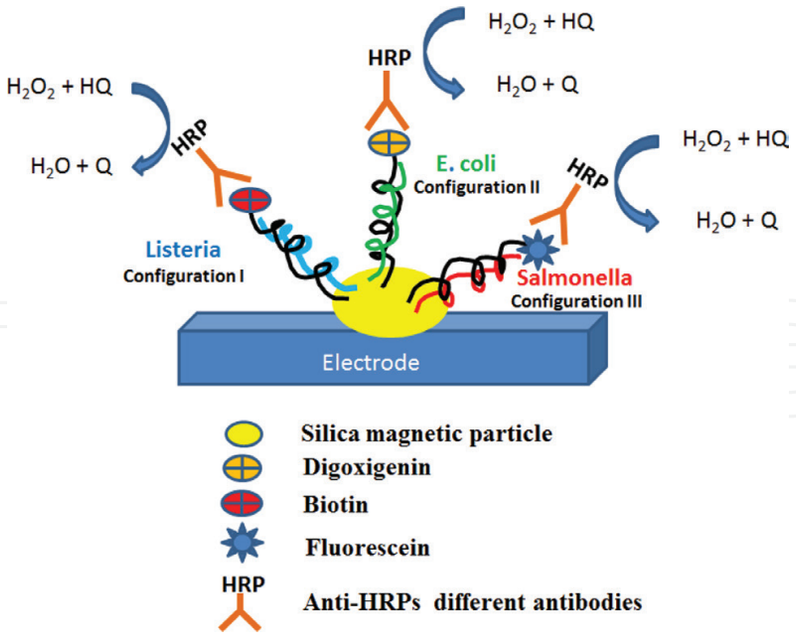

Figure 2. The construction of three configurations of DNA-amperometric magneto-genosensors for the independent detection of *S. enterica* Typhimurium LT2, *L. monocytogenes* DSM20600, and *E. coli* K12 strains. Type of used antibodies: AntiFlu-HRP (Anti-Fluorescein-Fab fragments), Strep-HRP (Streptavidin-POD conjugate), and AntiDig-HRP (Anti-Digoxigenin-POD Fab fragments). HQ-hydroquinone; Q-quinone. All bacterial strains were grown in Luria Bertani (LB) broth or agar plates for 18 h at 37°C.

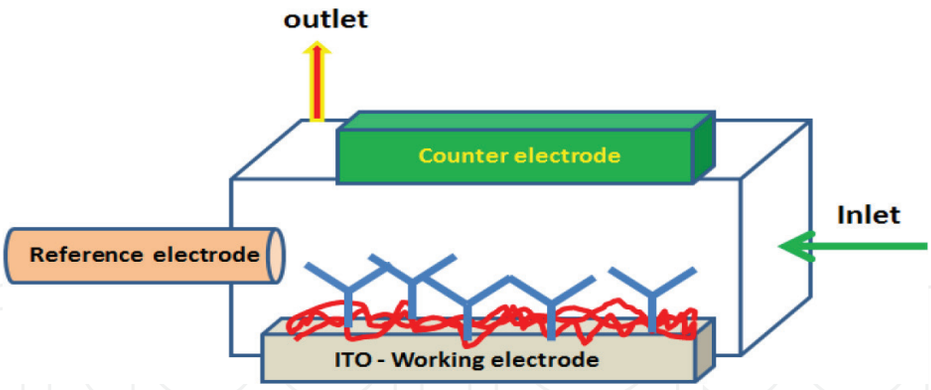

Figure 3. Electrochemical cell l using silanized ITO-electrodes and antibodies for specific detection of *S. typhimurium* and *E. coli K12* strains.

Impedimetric gold screen printed electrodes (AuSPEs) modified with thiolated *E. coli* antibodies were fabricated for the detection of *E. coli* at 10 CFU/mL level in river and tap water samples [24]. Another example of using EIS principles for specific detection of *E. coli* O157:H7 on ITO-based interdigitated microelectrode array in the presence of ($[Fe(CN)6]^{3-/4}$) electroactive redox probe (**Figure 4**) was also reported. Thus, it was found a direct correlation between the electron transfer resistance of the electrode (27.8%) and the concentration of *E. coli* cells (2.6 x 10^7 cells) upon their binding onto antibodies-based ITO array [26].

In another work, epoxysilanized ITO glass-modified with anti *E. coli* antibodies were used for EIS and atomic force microscopy (AFM) detection and topography investigations in the presence of different concentrations of *E. coli*. The research team found that *E. coli* cells and insoluble

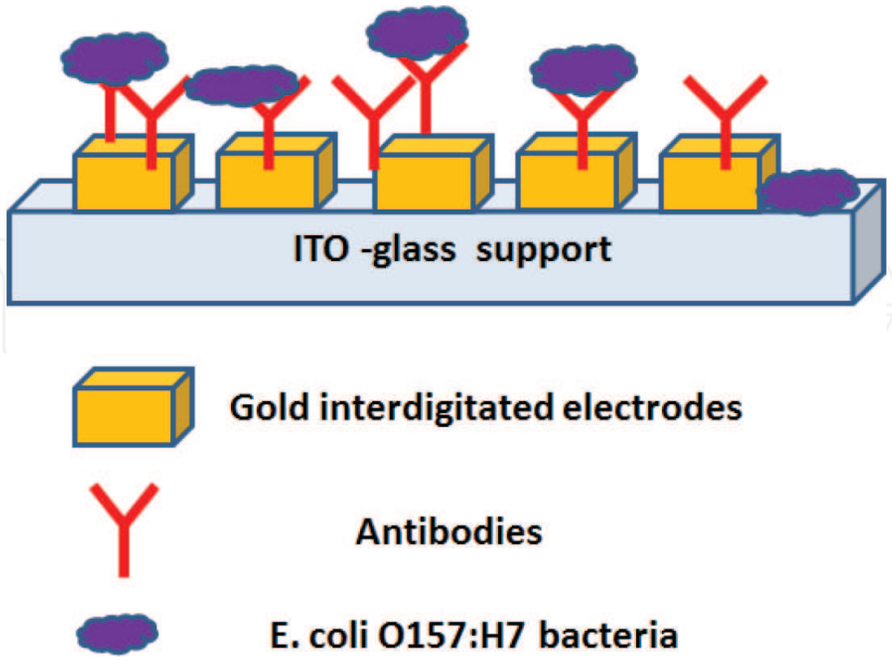

Figure 4. Direct impedance measurements using interdigitated immunosensors on ITO-glass support in the presence of ($[Fe(CN)6]^{3-/4}$) redox probe for the detection of *E. coli* O157:H7 cells.

precipitate mainly affected the electron transfer resistance and Warburg impedance even though the (3-glycidoxypropyl)trimethoxysilane chemical formed after immersion protocol over night at room temperature was uniform, dense, and homogeneous SAM monolayer onto ITO substrate. In this study, an enzyme labeled secondary antibodies namely alkaline phosphatase labeled secondary antibodies and its specific substrate 5-bromo-4-chloro-3-indolyl phosphate disodium were used for signal amplification of antibody bacterial interactions (**Figure 5**) [27].

Recently, homemade graphene-based field effect transistor (FET) Si/SiO_2—sensors for recording in real-time the proportional bias current signal responses in the presence of three different concentrations (1.6×10^7 CFU/mL, 1.67×10^7 CFU/mL, and 1.7×10^7 CFU/mL, respectively) of a nonpathogenic strain *E. coli* K12 ER2925 were reported [40]. Graphene FET biosensors were also developed to detect *E. coli* by recording the proportional increases in conductance

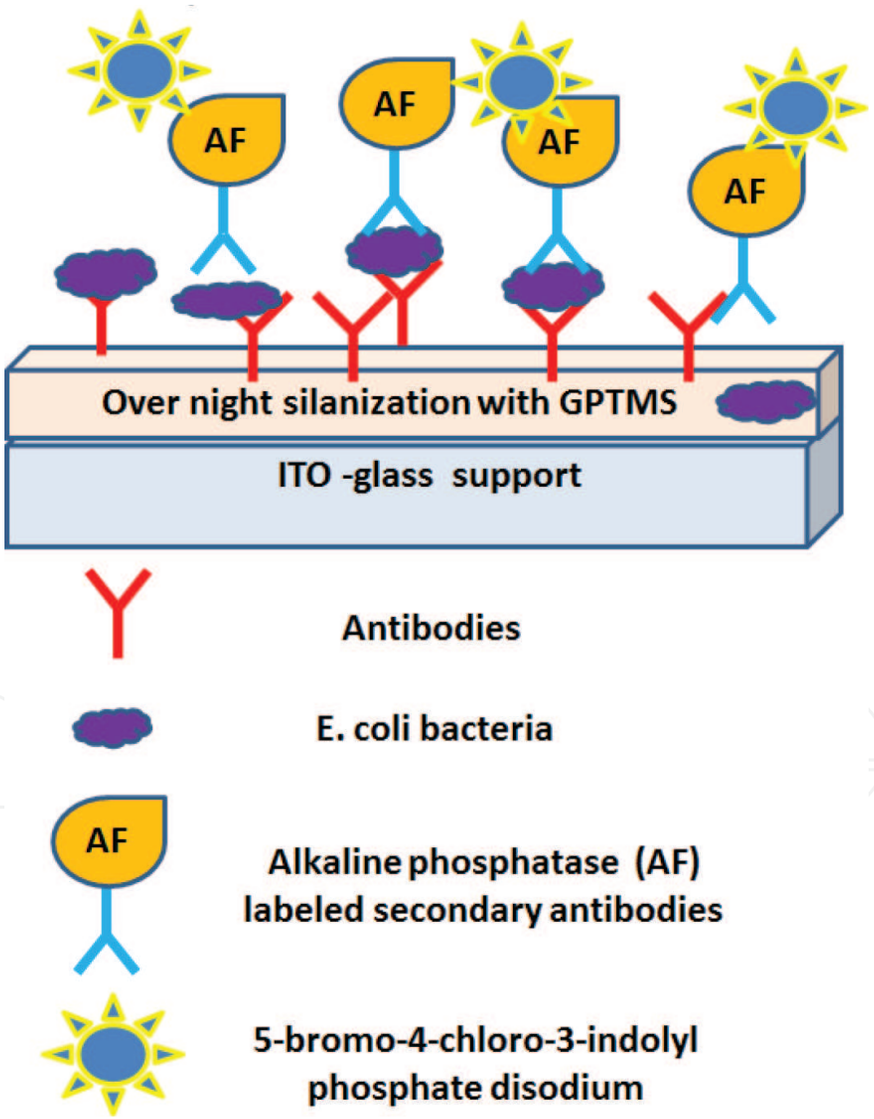

Figure 5. Silanization of ITO substrates with 3-glycidoxypropyl)trimethoxysilane (GPTMS) for further biofunctionalization with anti-*E. coli* antibodies.

values for different bacteria concentrations ranging from 0 to 10^5 CFU/mL. Artificial neural network and support vector regression algorithms have also been proposed for the *I-V* characteristics [41].

2.2. Optical biosensors

Fluorescence detection of several biomolecules in parallel using a sheath flow device with one inlet and five outlets has been reported [42]. On the other hand, it has been reported on the successful use of annealed (150°C for 4 h)/ultrasonicated mixtures of aqueous solutions of gold nanoparticles (Au-NPs ranging from 40 to 100 nM), and oxidized multi-walled carbon nanotubes (MWCNTs) for bacterial adhesion investigations of four different concentrations of *E. coli* namely 10^2, 10^3, 10^4, and 10^5/mL, respectively. The authors concluded that for increased contents of Au-NPs in composites, the Raman signals for higher *E. coli* concentrations improved, as bacteria have more Au-NPs to attach due to the transfer of its negative charge to MWCNTs [43]. Interestingly, bacterial anti-adhesive materials named "Rice leaf-like surfaces" (RLLS) (**Figure 6**) inspired by the hollowed morphology of rice leaves (*Oryza sativa* L. ssp. *japonica* cv. Calmati-202) were prepared on SiO_2-quartz glasses by a templateless, self-masking reactive ion etching approach with high optical-grade transparency properties (i.e., ≥92% transmission). The anti-adhesion property of *E. coli* O157:H7 during the dynamic flow conditions onto RLLS substrates was validated through fluidic channels at low flow (shear) rates and transmission spectra investigations at a wavelength range of 400–800 nm [44].

Label-free optical biosensors based on nanostructured (~50–100 nm) porous silicon ($PSiO_2$) thin films modified with synthetic peptide sequence K-7α12 OAKs (similar name K-$[C_{12}K]_7$, with K-lysine, and oligomers of acylated lysines-K) as a novel capture probe for whole bacteria *E. coli* ATCC 8739 and its lysates detection were reported [28] (**Figure 7**). Control experiments with neat and thiol-modified $PSiO_2$ (with no OAKs) exposed to lysate suspension of *Listeria innocua* and *Erwinia carotovora* (10^5 cells/mL) were also conducted to eliminate the possibility of non specific adsorption of bacterial lysate to the silicon-based surface. The same research team concluded that with K-7α12 OAK-tethered $PSiO_2$ substrates was possible to

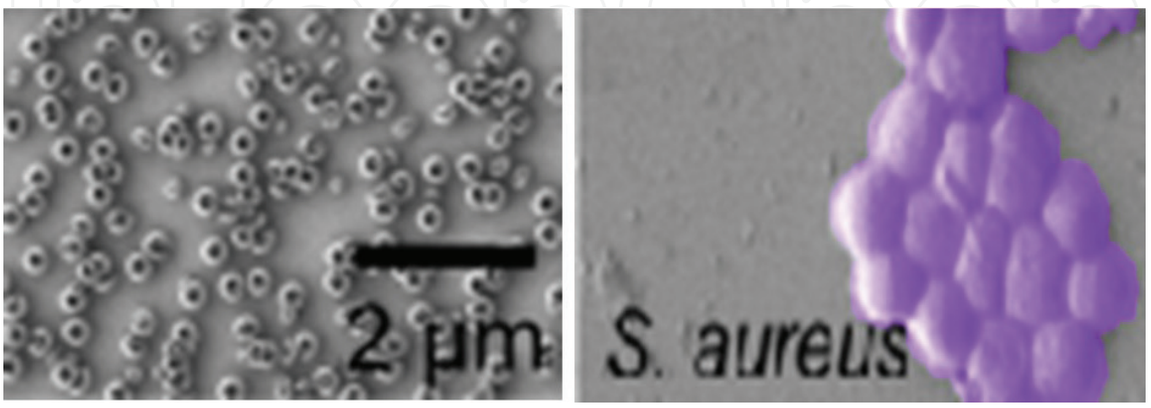

Figure 6. Bacterial (anti)adhesion on RLSS—substrates (a) and bacterial (*S. aureus*) adhesion on hydrophobic quartz (b). Reprinted with permission from Ref. [44] Copyright (2015) American Chemical Society.

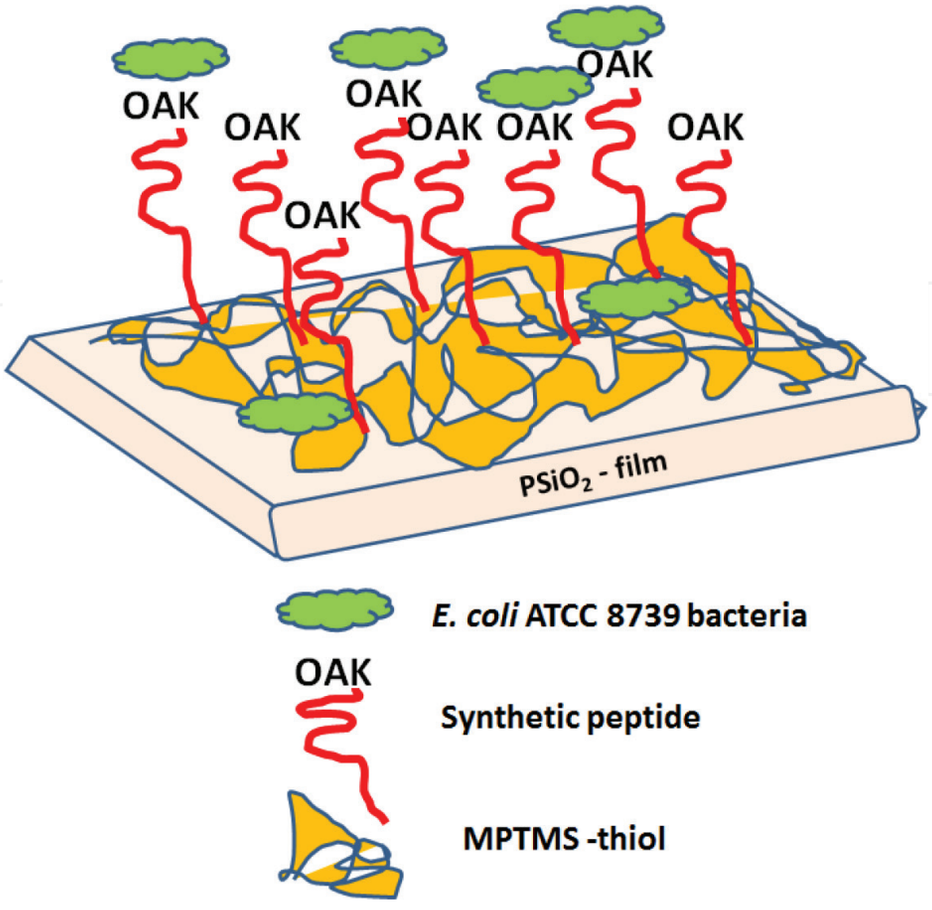

Figure 7. OAK-modified porous silicon ($PSiO_2$) modified with the synthetic peptide sequence K-7α12 OAK for the detection of *E. coli* ATCC 8739 and its lysates. MPTMS - mercaptopropyltrimethoxysilane.

achieve one order of magnitude improvement in the low detection limit in comparison with a previous team studies using monoclonal antibodies [45].

The ability of ssDNA probe for the *lacZ* gene of *E. coli* K12 (25 mer) covalently immobilized onto HEG/GOPS-silanized fused silica optical fiber (400 μm i.d. × 48 mm length) with a strong hybridization in the presence of its fully complementary target 10 pmol cDNA *E. coli* (25 mer *lac*Z), and ethidium bromide intercalator was reported. For systematic (bio)functionalization steps, the fibers were inserted into a holder system with cylindrical bores that accommodated maximum eight fibers at the time. Moreover, PCR amplicons of 100 m length containing the fragment of the *lac*Z sequence, and genomic DNA from *E. coli* were optically investigated. However, the authors mentioned that the optical system was not optimized for sensitivity whereas a significantly faster rate of non selective adsorption of non complementary oligonucleotides (25 mer to approximately 600 mer—ncDNA) than hybridization of complementary oligomers (cDNA) was recorded [1].

2.3. Acoustic biosensors

Commonly used acoustic wave biosensors are based on a thickness share mode (TSM) device [46] known as quartz crystal microbalance (QCM), which are classified as bulk acoustic

wave (BAW) devices. Thus, since 1980, various chemical and biological BAW sensors for the detection of either organic solvents or biomolecules have been reported [47]. Moreover, shear horizontal surface acoustic wave (SH-SAW), surface transverse wave (STW), love wave (LW), flexural plate wave (FPW), shear horizontal acoustic plate mode (SH-APM), and layered guided acoustic plate mode (LG-APM) have demonstrated a high sensitivity in the detection of biomolecules in liquid media [48]. *E. coli* O157:H7 cells were also detected using shear horizontal surface acoustic wave IDTs sensors connected to a computer aid design (CAD) software [49].

A functional mannose self-assembled monolayer in combination with lectin concanavalin A (Con A) for the detection of *E. coli* W1485 using a QCM as transducer was reported [50]. The multivalent binding of Con A to the *E. coli* surface O-antigen favors the strong adhesion of the bacteria to the mannose-modified QCM surface. The minimal detection threshold was 7.5×10^2 CFU/mL.

Air samples containing four biological warfare agents including *E. coli* MRE 162 (collected using a "cyclone" sampler (Biotrace International, UK) and concentrated in collecting buffer PBS Tween—0.01% v/v) were detected in parallel using a flow-through system with four piezoelectric QCM crystals biofunctionalized with polyclonal antibodies located in separate fluidic chambers in the first attempt to determine the low level of detection as well as the specificity of each agent binding to its specific immobilized antibodies [34].

SH-SAW devices fabricated on langasite (LGS, $La_3Ga_5SiO_{14}$) - crystal sputtered with chromium/gold layers accommodated interdigitated transducers (each IDT consisted of 240 electrodes) and a gold platform region for subsequent (bio)functionalization steps with cysteamine/NHS-PEG-biotin/Avidin/Biotinylated polyclonal rabbit immunoglobulin G (IgG) antibody directed against fluorescently labeled *E. coli* were imaged with a cooled CCD camera on a BX51 fluorescence microscope. Furthermore, The MetaMorph software was used for counting the cells and analyzing their selective binding to anti-O157:H7 LGS-slide versus non selective binding to an anti- trinitrophenyl hapten modified LGS-slide (control experiments) [35].

A simple and rapid method (less than 90 min) that combined the bulk acoustic wave (BAW) technique based on Ag-plated AT cut 9 MHz quartz crystal (diameter 12.5 mm) with the gelation reaction of *tachypleus* amebocyte lysate (TAL), was used for viscosity and density measurement, of *Escherichia coliform* in very small mixed volumes (100 μL) was reported. A thermostat was used to control the reaction temperature at 37°C through a thermostatic water jacket. Thus, the frequency decreases very slowly after an initial lag time according to the progress of gelation of TAL, then drops quickly and a sudden change (its mechanism is still under investigation) followed, with finally a constant value after the completion of the gelation. *E. coli* of unknown concentration was determined using a regression equation. The linear range of detected *E. coli* was recorded for 2.7×10^4–2.7×10^8 cells/mL [51].

Microcontact imprinted QCM crystal and SPR glass with *E. coli* cells were obtained by a sandwich approach based on HEMA/EGDMA/deionized water/MAH/the initiator AIBN containing stock monomer solution. A 3 μL aliquot was taken from the stock monomer solution and dropped onto the allyl mercaptan modified SPR and QCM chips. Thus, the limit of detection (LOD) and the limit of quantification (LOQ) were found as 3.72×10^5 CFU/mL and 1.24×10^6

CFU/mL with QCM system whereas 1.54 × 10^6 CFU/mL and 5.13 × 10^6 CFU/mL with SPR system (**Figure 8**). The microcontact imprinted QCM crystal and SPR glass selectivity and specificity were proved in the presence of *Bacillus* and *Streptococcus* of 0.5 McFarland (1.5 x 10^8 cells). *Bacillus* was selected due to its morphological similarity to *E. coli* whereas *Streptococcus* as a gram-positive bacteria. The authors concluded that both sensor surfaces have the ability to recognize *E. coli* with high affinity due to the obtained recognition cavities via microcontact imprinted glass with *E. coli* [52].

2.4. Microfluidic biochips

From the beginning, microfluidic biochips or lab-on-chip biosensors strongly attract interest of different research communities (physics, chemistry, biology, and business entrepreneurs) due to their undeniable advantages in terms of measurements in real environments of tested biomolecules, low volumes of bioreagents, small variations of temperature, and low cost of fabrication of flow cell usually based on poly(dimethylsiloxane) (PDMS) material that can be disposable. Several biological applications have been reported based on microfluidics including studies. Some are discussed in this section.

Poly(lactic acid) (PLA)/PLA-PEG–based electrospun nanofibers (NFs) and K3-Brij76 (KB) polymer were used for colorimetric single-step paper-based lateral flow assays (LFA) for the rapid detection of 1.9 × 10^4 cells *E. coli* O157:H7 bacteria through a well-known sandwich configuration: HRP-labeled secondary antibodies/streptavidin-conjugated sulforhodamine B (SRB)–encapsulating liposomes/anti *E. coli* captured antibodies adsorbed onto PLA-PEG NFs (**Figure 9**) [53].

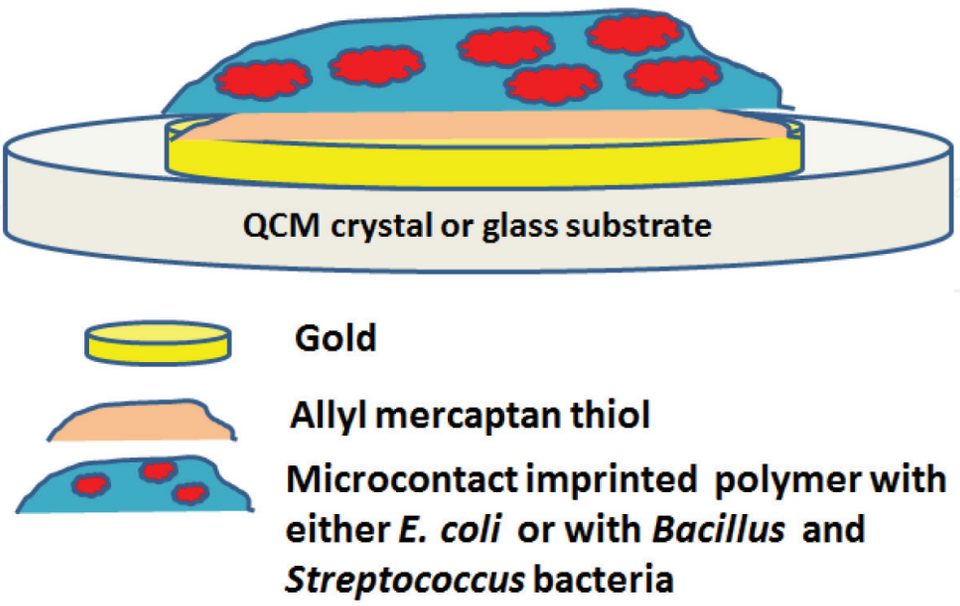

Figure 8. Surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) sensors using microcontact imprinted polymer with either *E. coli* (for specific detection) or *Bacillus* and *Streptococcus* (for non-specific detection).

http://dx.doi.org/10.5772/67392

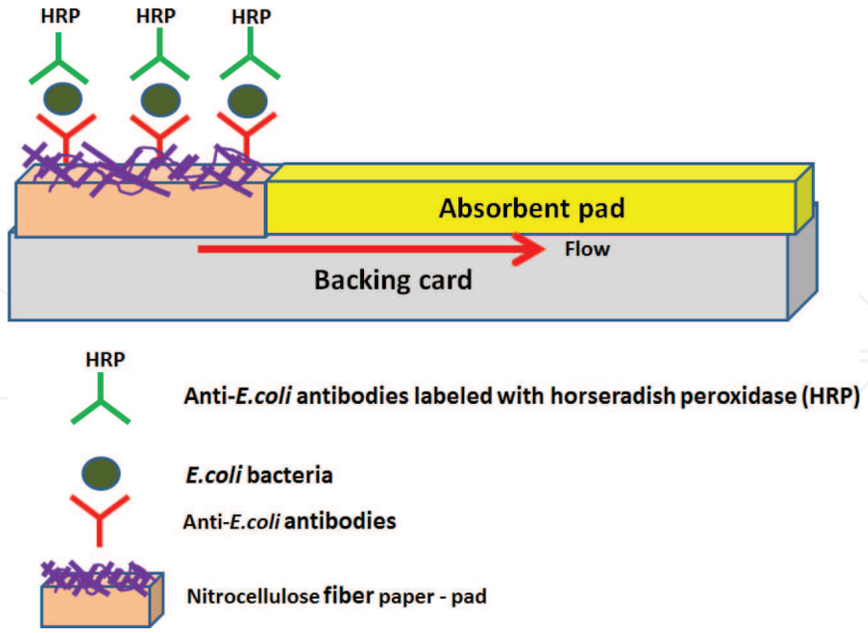

Figure 9. Colorimetric detection of *E. coli* bacteria using lateral flow (LFA) principles on nitrocellulose paper. In the absence of *E. coli,* the HRP antibodies flow through the nanofiber pad and no optical signal is observed.

A high-throughput PDMS microfluidic system with seven parallel channels (each channel contains 32 square-shaped microchambers) was employed for long-term growth monitoring of *E. coli HB101* (2.0×10^9 CFU/mL) growth monitoring and for the studies of inhibitory effects of various concentrations of two antibiotics (tetracycline and erythromycin at 0–4 μg/mL and 0–8 μg/mL, respectively) over bacterial cells. For flow antibiotic **(Figure 10**) sequential measurements were used bacterial suspensions at their stationary-growth phase. It was found that in the presence of at least 3 μg/mL tetracycline or erythromycin, the *E. coli* morphology remained similar to that of normal bacterial growth states cultured

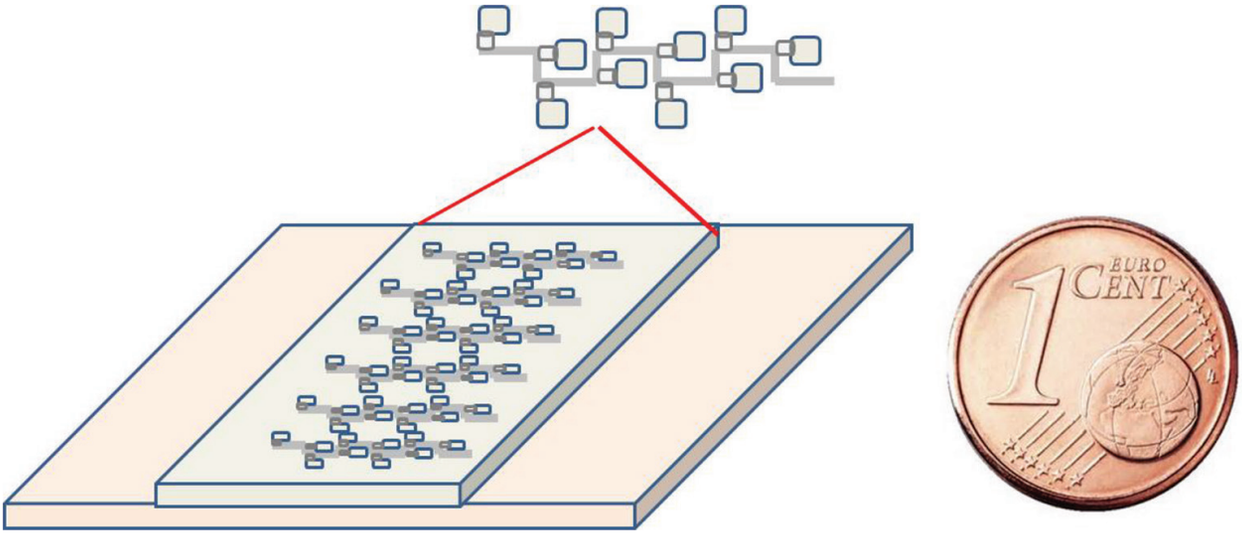

Figure 10. Configuration of the microfluidic device with multichannels.

in the absence of antibiotics, whereas in the presence of 3 μg/mL tetracycline for 8 h bacteria became filamentous [54]. Moreover, the mechanism of formation of long filamentous bacteria in the presence of cephalexin antibiotic was also studied with molecular biology techniques [55].

In June 2016, it was reported the fabrication of tortuous-shaped giant magnetoimpedance (GMI) sensor (working frequency 2.2 MHz) integrated into a microfluidic device (MFD) (**Figure 11**) using a homemade gold nanofilm biofunctionalized with monoclonal anti *E. coli* antibodies, *E. coli* bacteria/biotinylated polyclonal antibodies/streptavidin-labeled superparamagnetic beads (2.8 μm) and sensitive and specific detection of different concentrations of *E. coli* O157:H7 (50–500 CFU/mL) [54].

Electrochemically etched porous silicon (pSi) with ordered nanopore array integrated into a microfluidic PDMS channel was used for reflectivity effective optical thickness/fluorescence detection of specific *(E. coli,* ranging from 10^3 to 10^7 CFU/mL) and non specific (NOX and P17 strains) bacteria after their staining with a mixed solution of SYTO9 and propidium iodide, and confirmed by a significant pore blockage (specific) or any pore blockage (non specific) effects [30].

Sputtered nanofibers (NFs) of different densities (one layer sparse or larger pore sizes, one layer dense or smaller pore sizes, two layer sparse, two layer dense, three layer sparse, and three layer dense) with either positive or negative charge were tested for maximizing the amount of *E. coli K12* cells retained. For microfluidic investigations, nanofiber multilayers

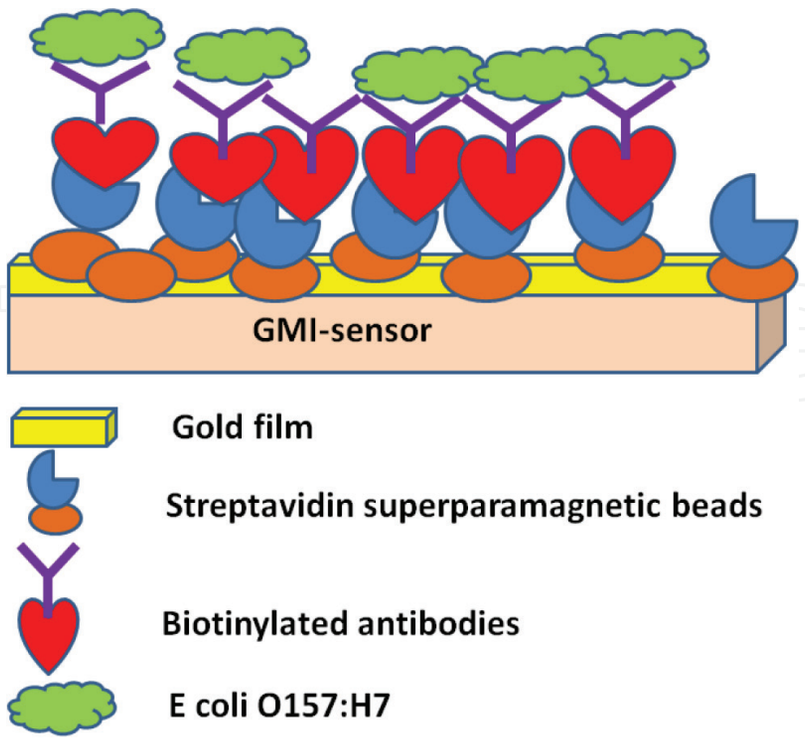

Figure 11. Tortuous-shaped giant magnetoimpedance (GMI) gold based-sensor for microfluidic detection of *E. coli.*

proved more homogeneous morphologies and were subsequently transferred onto poly(methyl methacrylate) (PMMA) films. Experimentally, negatively charged fibers spun with poly(methyl vinyl ether-alt-maleic anhydride) [PVApoly(MVE/MA)] of 300–400 nm and positively charged nanofibers of 450–550 nm have been employed. It was found that three layers sparse negatively charged NFs significantly reduced the non specific analyte (bacteria) retention (17%), whereas positive charged NFs were used for the detection of different concentrations of *E. coli K12* (87%). For this work, polyclonal anti *E. coli* antibodies were immobilized via EDC/sulfo-NHS chemistry on negatively charged NFs in order to selectively capture the negatively charged *E. coli* cells over 60 min using a syringe flow system and counted the number of colonies in the resulted effluent (low—average number of colonies in inlet solution was 62 CFU/mL) [57].

3. Conclusions

Paper microfluidics and smartphone technology were used for the detection of *E. coli* in real water sample using beads functionalized with anti *E. coli* antibodies predeposited in two out of a three channel paper device. It was found that by integration an internal gyroscope into a smartphone at an optimized angle of scatter detection, the presence of a single cell level in 90 s was possible [58]. Furthermore, a droplet-based, multiplexed fluorescence/light scatter submicron polystyrene particles functionalized with rabbit polyclonal anti K12 antibodies using nanofibrous substrate for the detection of 10^2 CFU/mL *E. coli* K-12 and *T. S. typhimurium* were reported in 2015. For experiments, all reagents were preimmobilized at fixed locations and included into two smartphones with necessary filters: one for incidence and the other for detection, positioned at 90° angle [59]. A microfluidic-cellulosic pad (μPAD) was loaded with polystyrene particles functionalized with polyclonal anti *E. coli* K12 antibodies and used for the detection of 10 CFU/mL *E. coli* K-12 in the human urine in about 30 s with a smartphone [60]. *E. coli* K-12 (10^1 CFU/mL) was detected in water sample (30 s) using also the principles of a μPAD-pad modified with antibodies coated particles and scattering intensity recorded by a smartphone with an autoexposure and autofocus locked on the central paper channel surface at 65° [61]. Identification of contaminated ground beef meat with various concentration of *E. coli* was possible without the need of functionalized particles with antibodies just by recording the resulted scattering light values at different angles with a smartphone after exposure to a perpendicular irradiation at 880 nm NIR-LED system. Therefore, different low detection limits were recorded: 10^1 CFU/mL at 45° and 10^2 CFU/mL at 30° and 60°. However, this method suffers from impossibility of distinguishing between different similar bacteria species (e.g., *E. coli* and *Salmonella* spp.) [62]. Moreover, the development of the first integrated paper-based DNA genosensor including nucleic acid extraction, amplification, and visual detection in about 1 h of *E. coli* (10–1000 CFU/mL) in spiked drinking water, milk, blood, and spinach using a smartphone was reported [63].

In conclusion, biosensors [62, 64, 65] and microfluidics [66] in combination with smartphone technology hold great hope that *E. coli* and various other coliforms may be detected in real time avoiding human suffering and save lives.

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