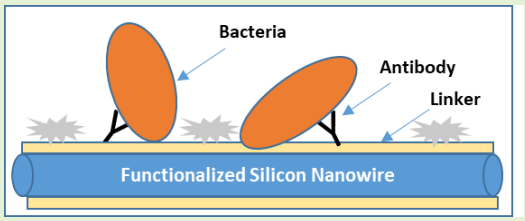
Recent Developments for the Detection of Escherichia Coli Biosensors Based on Nano-Objects-A Review

Yousra Benserhir, Anne-Claire Salaün, Florence Geneste, Laurent Pichon, Anne Jolivet-Gougeon

Recent Developments for the Detection of *Escherichia coli* biosensors based on nano-objects – A review

Yousra Benserhir, Anne-Claire Salaün, Florence Geneste, Laurent Pichon and Anne Jolivet-Gougeon

***Abstract*— Advances in nanotechnology have made it possible in recent years to develop strategies for rapid and sensitive detection of pathogenic bacteria using new nanomaterials and the development of electronic nano-sensors. The detection of bacteria still faces problems such as long analysis time and complexity of the process. An alternative method is the use of biosensors, which combines a biological recognition mechanism with a physical transduction technique. Thus, a number of methods and manufacturing technologies have been developed in order to achieve performance in sensitivity, detection limit, label-free detection or real-time analysis. This review aims to focus on the state of the art of biosensors for the recognition elements of Escherichia coli in label-free biosensors with a particular focus on the beneficial use of nanomaterials and nano-objects for detection. Among the recent related biosensors based on nano-objects for *E. coli* detection, the technologies and measurement techniques are detailed by comparing their performances detection in terms of concentration range and detection limit. Detection becomes more sensitive and more flexible using nanoparticles as markers, and real-time electrical detection methods are dominant in comparison with optical ones. The lowest detection limit can be achieved for sensors based on metal (gold or silver nanoparticles) with optical detection techniques in contrast with electrical detection methods using measurement conductance.**



***Index Terms*— Biosensor; nanosensors; nanomaterials; bacteria detection; functionalization; electrical detection**

I. Introduction

Infectious diseases have been historically ranked on top as one of the major challenges for human survival due to the periodical epidemic emergence of old and new outbreaks [1]. Foodborne infections are frequently transmitted by food contamination or cross-contamination during production, processing or transportation. Despite continuous efforts to improve safe-handling and processing practices, outbreaks caused by foodborne pathogens continue to arise, posing an imminent risk that needs to be effectively addressed. Biosensors have been proposed as rapid and cost-effective analytical methods to monitor bacteria in samples of interest. A biosensor is an analytical device which integrates a biological recognition element with a physical transducer to generate a measurable signal proportional to concentration of analytes. In the general scheme of a biosensor, the biological recognition element responds to the target compound and the transducer converts the biological response to a detectable signal, which can be measured electrochemically, optically, acoustically, mechanically, calorimetrically, or electronically, and then correlated with the analyte concentration. Different types of biosensors available as electrical, and optical devices can selectively detect biological species. A good biosensor must not only respond to low concentrations of analytes, but also have the ability to discriminate species based on the recognition molecules that are immobilized on its surface [1].

This review focuses on recent advances in the field of biosensors for the detection of pathogen bacteria and more specifically of *Escherichia coli*. Owing to the high interest of nanomaterials to enhance sensitivity and selectivity of biosensors, a special attention is made on the role of nano objects in biosensor. Thus, after a description of conventional methods for *E. coli* detection, alternative biosensor methods are presented with a brief reminder of the main transduction methods (optical and electrochemical). Then the role of nanomaterials or nano-object on biosensors performance is detailed and a comparison between the different biosensors is made in terms of concentration range and detection limit.

Manuscript received; revised; accepted. Date of publication; date of current version . The associate editor coordinating the review of this article and approving it for publication was .(Corresponding author: Anne-Claire Salaün). Y. Benserhir, A-C Salaün and L. Pichon are with the Institut d'Electronique et de Télécommunications de Rennes (IETR), University of Rennes1, Rennes, France (e-mail: asalaun@univ-rennes1.fr. F. Geneste is with Institut des Sciences Chimiques de Rennes (ISCR) (e-mail: florence.geneste@univ-rennes1.fr). A. Jolivet-Gougeon is with the INSERM, INRA INRAE, Institut NUMECAN (Nutrition Metabolisms and Cancer) (e-mail: anne.gougeon@univ-rennes1.fr).

II. Established methods for the detection of pathogens

A. Standard methods for in vitro diagnostic

In the bacteriology laboratory, many methods exist for detecting bacteria, whether based on direct examination, cultures, detection/quantification of antigens/ toxins or specific antibodies, use of animal models or methods of molecular biology.

Visualization of bacteria under an optical microscope (Gram or Ziehl stains, immunofluorescence, immunostaining ...) [2] often lacks sensitivity and specificity, unless the latter is improved by use of specific and labeled antibodies, (e.g. with a fluorochrome). The electron microscope allows a more precise visualization of bacteria, internal or external, but it is not very specific [3] and not extensively accessible. The detection of bacterial virulence factors can also be demonstrated by cell cultures, studying the cytopathogenic effect (toxicity), the capacity of adhesion and/or cell invasion, but remains in the field of research. Culture methods are the most commonly used, but their interest is limited for bacteria requiring a long incubation time or for viable non culturable bacteria (under stress ...), and null for bacteria for which no culture medium already exists. Quantitative or semi-quantitative measurements of bacteria by bacterial counts on agar media or flow cytometry [4] have also been proposed. Many methods based on the detection of antigens [5] or specific toxins [6] have been developed, in order to increase the specificity and selectivity of detection: agglutination of sensitized latex particles, rapid immunoenzymatic detection of antigens [7]. Another alternative consists in quantifying the serum antibodies (serology) generated by an in vivo stimulation by bacterial antigens [8]. This method is very specific according to the antigens used in vitro , and many tests exist (e.g. Enzyme Linked Immunosorbent Assay: ELISA…) [9], but are limited by the sensitivity often linked to the kinetics of appearance and disappearance of the antibodies, and the results of which may vary depending to the bacterial antigens and cross reactions [10]. Confirmation methods can be used to increase the specificity of the test, such as Western blot or immunoblot (different proteins are separated on a membrane which serves as a reaction support to specify against which specific proteins the antibodies are directed).

Numerous molecular biology methods have been developed, based on the detection of nucleic acids, often after molecular amplification, ranging from simple PCR (Polymerase Chain reaction) to recent NGS (Next generation Sequencing) methods [11]. Among these we can cite molecular hybridization and its variants (hybridization with signal amplification or bDNA), gene amplification or PCR/ multiplex PCR, DNA microarray [12], nucleotide sequencing and derivatives (NGS, syndromic panels ...). These methods made it possible to study whole microbiota under different conditions and compare them. Traditional methods of cloning a DNA sequence into a vector with replication in a living cell require days or weeks of work. The use of the PCR, in molecular diagnostics or research laboratories, is now accepted as the gold standard for detecting nucleic acids from a number of origins [13]. Different PCR methods exist, based on the RNA or DNA detection, whether qualitative or quantitative, in real time, simple or multiplex. These methods require small sample size and are extremely sensitive and rapid techniques [14], which do not require a bacterial culture step. Mutations in targeted sequence can lead to false negative results, and contamination (if the strict handling conditions are not respected) to false positive results. Real-time PCR analysis can be completed faster and simultaneously detect several species (multiplex), within several hours, but still requires specific equipment and reagents [15]. Gadsby *et al* [16] detected eight bacterial targets that could be reliably quantified from sputum samples by two real-time PCR assays.

These methods still face the issues such as extensive analysis time and process complexity. An alternative method for the detection of microorganisms is the use of biosensors, which combines a biological recognition mechanism with a physical transduction technique. In this way, number of methods and technologies of fabrication have been developed to aim the following performances (but difficult to meet simultaneously): high sensitivity, low detection limit, simultaneous detection and identification of different strains, real-time analysis, and especially portability and an ease of use for in-situ monitoring.

B. Optical biosensors

In comparison with biology detection methods previously mentioned, optical biosensors offer some possibilities for ease of use, rapid, portable, multiplexed, and cost-effective diagnosis. In this part, we review recent advances in optical biosensors for pathogen diagnosis, especially colorimetric and plasmonic sensors, and introduce technologies and strategies that constitute those optical biosensors with such sensing performances. Recent examples of optical biosensors are presented and their advantages and limitations are discussed.

Optical transducers represent a board class of common and popular signaling methods, all involving an optical change in the sensing layer, including absorption, transmittance, scattering, reflection, refraction, and emission, as a result of interactions between the analyte and the recognition element. Optical sensors are commonly based on colorimetry, UV absorption, fluorescence, chemiluminescence, surface-enhanced Raman scattering (SERS), or surface plasmon resonance (SPR), which can be detected by the naked eye or an optical sensing instrument, with the advantages of versatility, no destruction, sensitivity, universality, and simultaneous detection of multiple targets [17] [18]. Several research teams have worked on the development of this type of sensor using substrates, based on materials that can be at the nanometer scale, for examples: paper, glass slide, polymer, multilayer on glass substrate, polystyrene microparticle, gold nanoparticles (AuNPs), gold nanowires (AuNWs), carbon nanotube, Au plate [19]. Colorimetric biosensors have attracted wide attention owing to their easy readout and fast visual detection through the naked eyes or low-cost and portable equipment, which can be used to detect the analytes based on the color variation [20][21]. Thus, Ji et.al. [22] developed a colorimetric sensor with positively-charged amine-terminated polyamidoamine

dendrimers to capture bacteria and reported a detection limit of 1.0×10^4 cells/mL. Other teams have validated the detection of proteins [23], bacteria [24], viruses [25], and cancer cells [26], with functionalized gold nanoparticles. In 2013, the hybrid systems that consist of Au NPs and surface-assembled fluorescent probes were exploited for fluorescent turn-on sensing of liposaccharide (LPS). The results showed that the sensitivity and selectivity to LPS relied strongly on the binding affinity between fluorescent probes and Au NPs [27]. Remaining in 2013, Zagorovsky *et al* [28] used, for DNA detection, a colorimetric sensor modified with gold nanoparticles (GNPs) and this study showed a detection limit of 500 pM pathogenic DNA. The success of GNPs ranging in size from 1 nm to several hundred nanometers is due to their strong light-scattering properties. The intensity of light scattering is based on the size of the particle. Moreover, GNPs can be easily conjugated with protein(s) or modified DNA molecule(s) through sulfhydryl linkages. These properties make GNPs a useful tool for ultrasensitive molecular detection [29]. One of the notable recent trends in biosensors is the development and application of plasmonic biosensors for pathogen detection. Two major plasmonic biosensors use surface plasmon resonance (SPR) and surface enhanced Raman spectroscopy (SERS), stable and sensitive detection tools that can detect low bacterial concentration [30]. The detection of Escherichia coli (*E. coli*) O_{157}:H_7 by a surface plasmon resonance sensor (SPR) used three sample preparation methods of bacteria: untreated (viable), heat-killed and then soaked in 70% ethanol, and detergent-lysed. The SPR detection surface consisted of a monoclonal antibody immobilized on a monolayer of self-assembled alkanethiols (SAM) terminated with -COOH and -OH on a gold surface. The limit of detection (LOD) of each method is determined by the minimum measurable shift in resonance wavelength corresponding to the specific binding of *E. coli* O_{157}:H_7 and subsequent binding of an antibody for amplification. Detergent-containing samples produce the lowest detection limit at 10^4 CFU/mL, while the detection limit was 10^5 CFU/mL for heat-killed samples and 10^6 CFU/mL for untreated samples, respectively [31]. A fibre-optic sensor was developed and optimised for the simultaneous detection of *E. coli*, Listeriaand Salmonella in food. Streptavidin-coated optical waveguides were immobilized with biotinylated polyclonal antibodies and exposed to bacterial suspensions or enriched food samples for 2 hours, allowing a detection limit of 10^3 CFU/mL [32].

C. Electrochemical biosensors

Electrochemical biosensors have been intensively studied and well developed for the detection of pathogenic bacteria. Some advantages of electrochemical biosensors over others include comparable sensitivity, low cost, fast response, and possibility to operate in turbid solution and to be miniaturized. According to the variable measured, these electrochemical biosensors can be classified into three classes: amperometric, potentiometric, impedimetric biosensors, for current, potential and impedance/capacitance measurement, respectively.

1) *Amperometric biosensors*

One of the most uses electrochemical detection techniques is the amperometric detection. Amperometric sensors monitor the changes in current or potential caused by the oxidation or reduction of the electrochemically active analyte in the electrochemical system. The redox reaction directly or indirectly involves the analyte, and a linear relationship between the analyte concentration and the measured current can be observed [33], [34].

The following examples illustrate the different methods of manufacturing amperometric sensors for *E. coli* detection, as well as their detection limits. The development of an amperometric biosensing platform for the detection of pathogenic *E. coli* O_{157}:H_7 has been proposed by Dhull *et al*. [35]. The biosensor developed works on the principle of antibody-antigen interactions, where the antibody is covalently bound to the surface of the nickel oxide thin-film matrix prepared by sputtering. The sensors have a wide linear range of 10^7 to 10^1 cells/mL with a significantly low detection limit of 1 cell/mL. The biosensors were found to be highly specific and selective towards the target species, even in samples composed of several bacterial species.

In another study, a method for easy detection of *E. coli* has been established based on the enzymatic activity of intracellular β-D-galactosidase (Gal) [36]. The bacteria was first treated with a gene expression inducer (isopropyl-β-D-thiogalactopyranoside IPTG), leading to the increase of the expression level of Gal inside *E. coli*. Then, the hydrolysis of 1-naphthyl-β-D-galactopyronoside into 4-aminonaphthol by Gal occurred, facilitating by a permeabilization treatment of the bacteria. The electrochemical detection of 4-aminonaphthol amplified by an electrochemical-chemical redox cycling led to a detection limit of 10^5 CFU/mL for 30 min of IPTG treatment. By this method, a detection limit of 1.0 CFU/mL was reached with a 4-hour IPTG treatment, without DNA amplification or immunoassay protocol [37].

Another amperometric immunosensor has been developed for the rapid detection of heat-killed *E. coli* O_{157}:H_7 by Yan Li *et al*. [38]. This immunosensor was prepared as follows. First, long chain, amine-terminated 11-amino-1-undecanethiol alkanethiol hydrochloride (AUT) was self-assembled on a gold electrode surface to form an ordered, oriented, compact and stable monolayer with -NH2 functional groups that could immobilize massive gold nanoparticles. Then successive layers of AuNPs and a nanocomposite formed by the combination of chitosan–multiwalled carbon nanotubes and SiO2/thionine nanoparticles were added. Finally, *E. coli* O157:H7 antibody (anti-*E. coli* O157:H7) was covalently bound to the AuNP monolayer and its bioactivity was measured by amperometry. Under optimal conditions, the calibration curve for *E. coli* O157:H7 had a working range of 4.12×10^2 - 4.12×10^5 CFU/mL, and the total analysis time was less than 45 minutes.

2) *Potentiometric biosensors*

Potentiometric biosensors use a high voltmeter to measure the electrical potential difference or electromotive force between the working and reference electrodes. The

electrochemical process is non-faradic, with zero or negligible current in the electrochemical cell, and the potential difference is generated by the accumulation of charge density on the surface of working electrode. A Spanish team has demonstrated that the detection and label-free identification of live bacteria in real complex samples can be performed in few minutes and directly, by a simple and selective method, using an aptamer-based potentiometric biosensor. This biosensor used signal-walled carbon nanotubes as excellent ion to electron transducer and covalently immobilized aptamers as biorecognition elements. A highly selective detection of live bacteria with an immediate linear response of up to 10^4 CFU/mL was validated with a detection limit of 6.0 CFU/mL [39]. In the same context a potentiometric sensor was developed by Tian *et al*.[40]. This is a light-addressable potentiometric sensor (LAPS) deposited with highly oriented ZnO nano-arrays, used for the label-free detection of signal-stranded bacterial DNA. For sensitive detection of *E. coli* O157:H7 DNA, surface functionalization was achieved using covalent immobilization of the single-stranded DNA (ssDNA) of the specific probe on the sensor surface. The results indicate that

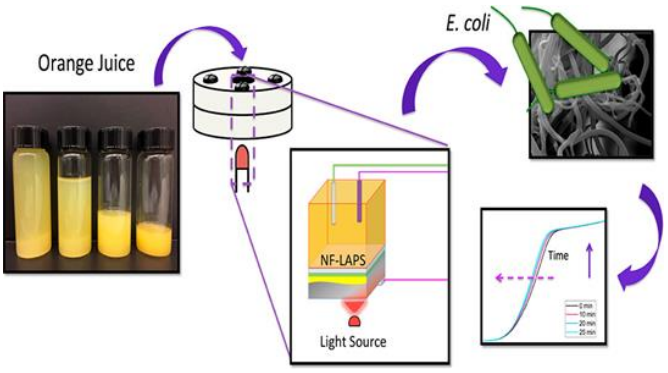


Fig. 1. Schematic representation of the NF-LAPS sensor comprising a three-electrode system. From Shaibani *et al*. [41], reprinted with permission.

distinct signal changes can be registered and recorded to detect the target *E. coli* ssDNA in the range concentration 10-10^5 CFU/mL. The lower detection limit of the target ssDNA corresponded to 1.0×10^2 CFU/mL of *E. coli* O157:H7 cells. Shaibani *et al*. [41] developed sensors for *E. coli* detection in orange juice, using a portable nanofiber-light addressable potentiometric sensor (LAPS). They chose a pH-sensitive electrospun poly(vinyl/alcohol)/poly (acrylic acid) (PVA/PAA) hydrogel nanofiber functionalized with D-mannose as sensitive layer. This sensor enabled real-time detection of the bacterial species in less than one hour, with a detection limit of 10^2 CFU/mL. A high selectivity of the biosensor for *E. coli* against the sugar fermenting Salmonella Typhimurium (S. Typhi) was obtained, attributed by the authors to the presence of D-mannose on the surface of the nanofiber.

3) *Impedimetric biosensors*

a) Electrochemical impedance spectroscopy detection (EIS)

Impedance measurements have become increasingly popular as a label-free detection tool for many different types of biosensors, during the past few years. The biosensors based on impedance measurements are prepared on electrodes such that the electron transfer is selectively modulated by the analyte [42]. A highly sensitive electrochemical biosensor has been developed by Krupinska *et al*. [43] for the detection of *E. coli* in water. The detection

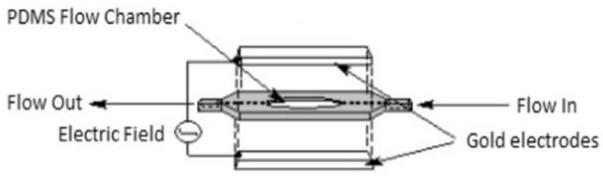


Fig. 2. Schematic picture of flow cell with two-electrode configuration and polydimethylsiloxane (PDMS) flow chamber. From Krupinska *et al*. [43], reprinted with permission.

system is manufactured using two gold electrodes in a flow cell modified with polyclonal antibodies against *E. coli*. The results are promising and show that label-free detection of trace amounts of *E. coli* is certainly possible with a simple and straightforward surface modification of the electrode.

Always with the same detection method, the immobilization of antibodies at the gold electrode was carried out through a stable acyl amino ester intermediate generated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydrosuccinimide (NHS), which could condense antibodies reproducibly and densely on the self-assembled monolayer (SAM). The resistance of the electrical transfer increases as a function of the immobilization of the antibodies and the adhesion of the *E. coli* cells at the electrode. This change is directly detected by electrochemical impedance spectroscopy in the presence of Fe(CN)63-/Fe(CN)64- as a redox probe [44]. In order to detect Gram-negative bacteria, a Brazilian team chose to work on bio-sensors with gold electrodes, functionalized with gold nanoparticles modified with cysteine, then they add clavanin A antimicrobial peptides. The modifications at the electrodes were evaluated by electrochemical impedance microscopy and cyclic voltammetry analysis [45] (figure 3).

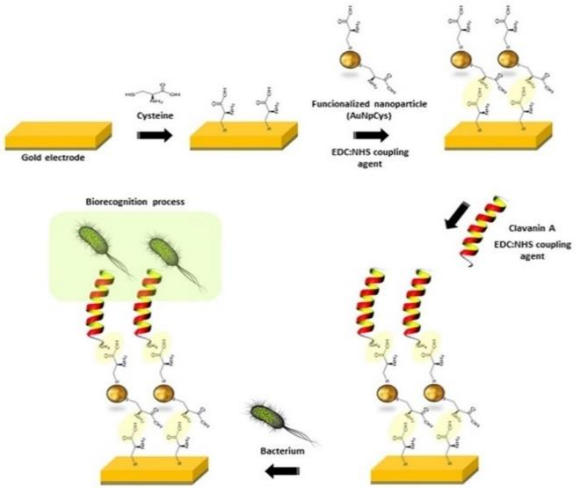


Fig. 3. Schematic representation on the functionalization steps including the cystein functionalization of gold electrode followed by the immobilization of functionalized gold-nanoparticles and then Clavanin A by coupling reactions. From De Miranda *et al*. [43], reprinted with permission.

The sensor successfully differentiates concentrations of *E. coli* ATCC 2522 between 10^1 to 10^4 CFU. Therefore, the proposed biosensor is a usable and simple alternative for bacterial detection with a possible use for quantitative detection.

b)Electrochemical capacitance spectroscopy detection (ECS)

Among electrochemical biosensors, capacitive biosensors show interesting properties due to changes in the dielectric properties of the electrode surface. Isil *et al* have shown that molecular printing is a well-suited technique with real-time detection of *E. coli* in the range of 10^2-10^7 CFU/mL [46].

The biosensors presented in this section demonstrated performance in terms of sensitivity, selectivity and reproducibility. Using different types of materials, researchers in this field have been able to demonstrate their optical, electrical or chemical capability for the detection of microorganisms.

III. NANOMATERIALS AND NANO-OBJECTS FOR THE DETECTION OF *E-COLI* BACTERIA

The use of nanoparticles and nano-objects increases in health and wellness, particularly for the detection or study of their effects on pathogenic bacteria. Due to their small size (<100 nm), nanoparticles have a larger specific surface area than the same materials on a larger scale. Quantum effects are important and can benefit for biosensing. In addition, below a size of about 20 nm, an increased surface reactivity appears due to surface energy [47]. It is this feature that gives them chemical reactivity and new physical properties (optical, mechanical, electrical and magnetic) that are of interest for interacting with biological systems, particularly in the case of biological species recognition.

Nanoparticles are used either for their ability to amplify the detection signal or for their biological actions on detection. In addition, the use of nanoparticles is compatible with the fabrication of ultra-sensitive, rapid, small and portable biosensors (e.g. usable as patches). In this way, nanoparticles contribute to the emergence of new detection techniques and also to new transduction methods. With regard to the detection of pathogens, efforts are focused on some properties of nanoparticles [48], [49]. There are several methods for the detection of biological species, including *E. coli*, using the specific properties of nanoparticles, of which the most common and most promising are electrochemical, optical, magnetic and gravimetric based detection methods.

On the other hand, recent advances in micro-nanotechnologies have led to the emergence of innovative devices with the integration of nano-objects such as nanowires (NWs), carbon nanotubes (CNTs) and nanomaterials in biosensors. The use of biosensors combining a biological recognition mechanism with a physical transduction technique is very promising because they are able to detect pathogens quickly and accurately. Due to their high surface-to-volume ratio, nanostructures are potential functional probes for detecting filler species as their dimensions are comparable to those of chemical and biological species. These technologies can be combined with specific sensitive layers to enable selective chemical detection. The miniaturization of biosensors offers several advantages, including reduced sample and reagent consumption and increased sensitivity with cost-effective disposable chips through mass production. These nano-objects as biochemical nano-sensors are promising for marker-free, real-time, biomolecule-sensitive detection. Their functionalization based on the chemical modification of the surfaces allows a more specific detection. They have shown great interest in the many biorecognition studies for biological analysis such as DNA, proteins, bacteria or viruses.

A. Electrochemical nanoparticles-based biosensors

The combined effects of the electronic properties and electrocatalytic activity of some nanoparticles can be a benefit for electrochemical detection (voltammetry, impedancemetry, amperometry...). Generally, these methods combine tools for the enhancement of the electrochemical signal, and recognition of biological elements.

For instance, anode material combined with nanoparticles binded to biological agent to target bacteria or acting as markers can be used in biosensors, and different detection signals can be obtained according to the detection method. In particular, recent work [50] reported the detection of *E. coli* using differential pulse voltammetry, integrating gold nanoparticles on the surface of a polypyrrole-reduced graphene oxide (PPy-rGO) composite for immobilization of a capture antibody (Ab1), and conjugated onto ferrocene doped polypyrrole-gold nanoparticles composite for immobilisation of detection antibody (Ab2) (figure 4). In this case, the attachment of Ab2 allows the generation of electrons by the oxidation of ferrocene. Due to a high surface area, good conductivity and biocompatibility of the nanocomposite, enzyme-free detection of *E. coli* K12 was shown, with a linear range from 1.0×10^1 to 1.0×10^7 CFU/mL and a low detection limit of 10 CFU/mL.

Another approach could be the use of anode material combined with nanoparticles to increase the surface area of the working electrode, and to improve the electron transfer, leading to higher electrochemical signal.

A recent study reported the photo-electrochemical detection of bacteria with zinc oxide (ZnO) nanowires modified with gold nanoparticles (AuNPs) and CdS quantum dots (CdS-QDs) as photo anode material [51].

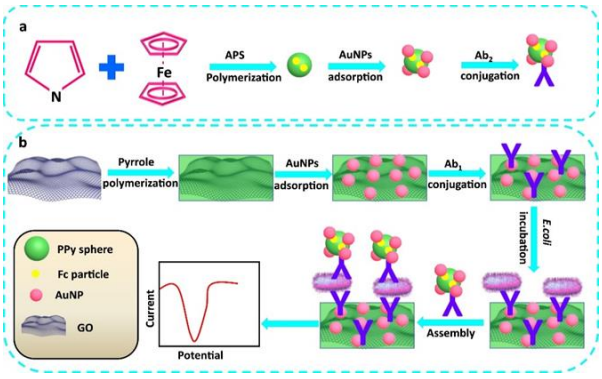


Fig. 4. Schematic illustration of: preparation of the PPy-rGO/AuNPs nanocomposite followed by Ab1 attachment and detection of *E. coli* K12. From Zou *et al.* [50], reprinted with permission.

Zinc oxide is a good semiconductor for photochemical detection biosensor [52], and its form as nanowires increases the surface of the electrode. The quantum dots promote the light absorption from UV to visible region and gold nanoparticles improve the electrons transfer. In the study reported by Dong *et al.* [51], *E. coli* O 157:H7 detection was ensured after assembling aptamer biological recognition element to the ZnO/AuNPs/QDs-CdS complex (figure 5). The results showed linear detection over a range from 10 to 10^7 CFU/mL with a detection limit of 1.125 CFU/mL. Such an approach is a good alternative for low noise, fast, and ultra-high sensitive detection of bacteria in comparison with the classical electrochemical detection.

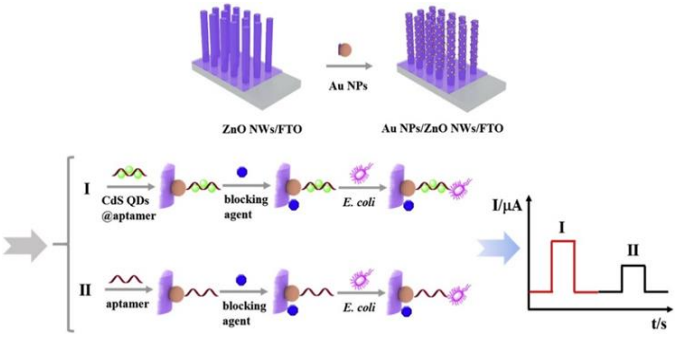


Fig. 5. Assembly process of the photoelectrochemical-biosensing for *E. coli* O157:H7. The process I states the PEC response based on aptamer/CdS QDs/Au NPNWs as a control. From Dong *et al.* [51], reprinted with permission.

B. Optical nanoparticle-based biosensors

The main optical detection methods of *E. coli* based on nanoparticles are colorimetry, fluorescence and surface plasmon resonance (SPR). The use of nanoparticles is particularly interesting for the detection of biological elements by the SPR technique, thanks to the presence of intense absorption peaks leading to an amplification of the detection, or to the change in refractive index at the sensor surface depending on analyte concentration and allowing to investigate the binding events of molecular interactions in real time and label-free. For example, a study has shown a reduction of the detection limit of *E. coli* by a factor of 5500 compared to a sensor without the use of gold nanoparticles [53]. More recently, highly selective *E. coli* SPR detection at low detection limit 0.57 CFU/mL in urine was reported using silver nanoparticles entrapped into imprinted polymer mixture to increase the sensitivity of the sensor (figure 6) [54]. For fluorescence detection semiconductor quantum dots (CdSe/Zn,...) combined with biological agent are used, due to their fluorescence properties of higher intensity than classical fluorophores [55][56]. In the case of quantum dots, it is possible to adapt their fluorescence emission spectra according to their size and composition [57][58], which makes them very attractive for the detection of bacteria. Moreover, thanks to the high selectivity of bioreceptors,

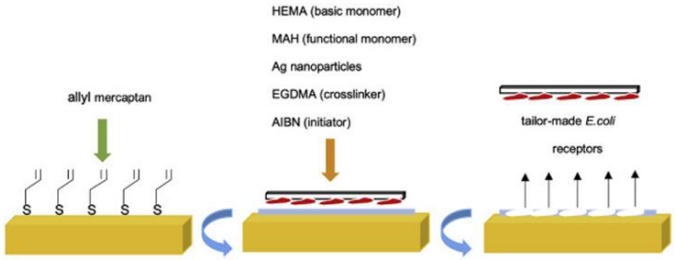


Fig. 6. Schematic representation of *E. coli* imprinted polymeric film synthesis via microcontact imprinting technique. From Özgür *et al*. [52], reprinted with permission.

multiple detection within the same sample could be possible [59], [60]. Recently, an original study reported *E. coli* detection on smartphone-based electrogenerated chemiluminescence (ECL) system using graphene quantum dots/silver nanoparticles complexes (GQDs/AgNPs) [61] (figure 7). In this case, graphene quantum dots are used to amplify and stabilize the luminescent signal, functional modules on smartphone for voltage excitation and luminescence capture, and luminescence image analysis. Based on this system, *E. coli* detection was demonstrated in a linear response in the concentration range 10-10^7 CFU/mL

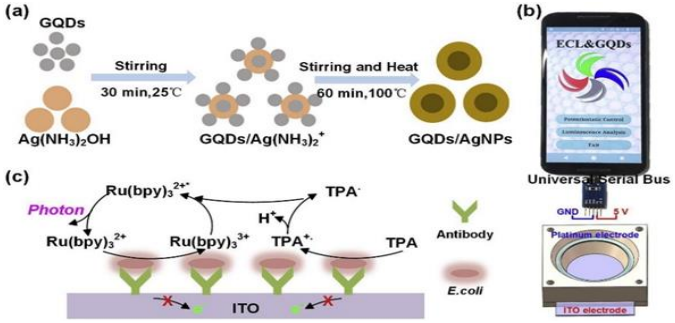


Fig. 7. Nanocomposites and system. (a) Preparation of the GQDs nanocomposites. (b) Smartphone-based ECL system with ITO electrode and platinum electrode. (c) ECL principle for *E.coli* detection. From Li *et al*. [61], reprinted with permission.

with a limit of detection of 5 CFU/mL.

C. Magnetic nanoparticle-based biosensors

Magnetic nanoparticles have specific properties (biocompatibility, non-toxic, and presence of functional groups) and are of interest for ultrasensitive biosensors and multiple pathogen detection. In addition, some hybrid magnetic nanoparticles provide various and effective detection methods thanks to the coupling of their magnetic properties to their electrical [62], [63] or their optical properties [64]–[66]. In particular, combination of magnetic nanoparticles with biological agents is an efficient strategy for identification, pre-concentration and separation of a bacterial strain in a given analyte through the application of a magnetic field. Magnetic nanoparticles can be used as homogeneous carriers for the capture of targets and the electrode as sensing element.

For example, combination of double antibody sandwich complex (magnetic bead-*E. coli* O_{157}:H_7 monoclonal antibody)-(*E. coli* O_{157}:H_7)-(enzyme-labeled anti-*E.coli* O_{157}:$H7$ antibody) has been used for the detection of *E. coli* O_{157}:H_7, using interdigited gold electrodes impedance system[60] (figure 8). The immobilization process has been performed with magnetic beads instead of electrode functionalization and sample absorption to improve reproducibility and sensitivity. In this case, detection limits ranging linearly from 10^2 to 10^6 CFU/mL has been achieved. Another work, reported *E. coli* detection in the milk in the range 5-10^8 CFU/mL with a LOD of 10 CFU/mL by fluorescence method combining carbon quantum dots synthesized from orange peel with magnetic nanoparticles (Fe_3O_4) prepared by simple, non-polluting, time saving and low cost method [65].

D. Gravimetric nanoparticle-based biosensors

Gravimetric detection method is based on a piezoelectric material (quartz, lead zirconium titanate-PZT…) microbalance whose principle is the measurement of the mass variation, related to the variation of the resonance frequency of the piezoelectric material correlated to the mass of the analyte deposited on the electrode of the microbalance. Thanks to the benefits of nanoparticles positioned on one of the balance electrodes, a better immobilization of the bioreceptors is possible, and thus an amplification of the detection signal and a better sensitivity of the sensor can be reached. Several studies have demonstrated the benefits using nanoparticles for the detection of *E. coli*, but few studies reported on their detection by gravimetric detection method. However, previous work has shown that it is

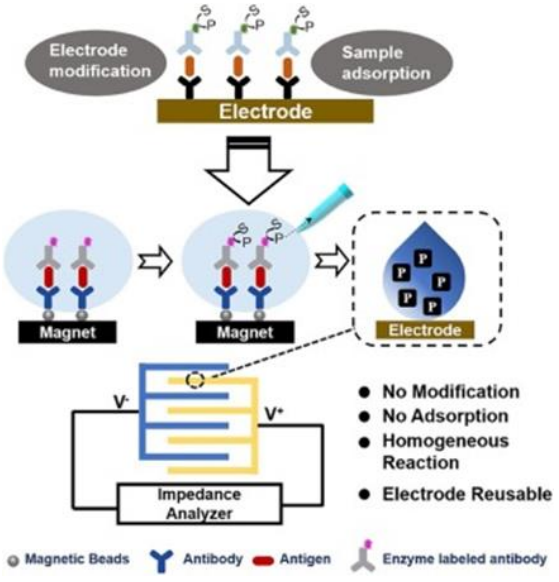


Fig. 8. Illustration of the magnetic bead-based impedance method for the detection of *E. coli* O157:H7, using interdigited gold electrodes. From Wang *et al*.[62], reprinted with permission.

possible to improve the performance of a biosensor for the detection of *E. coli* O_{157}:H_7 compared to a conventional sensor. It was due to the high and specific biotin-binding affinity of streptavidin to attach gold or iron oxide nanoparticles to the complementary DNA strand leading to detection limits of 2.0x10^3CFU/mL and 2.67x10^2CFU/mL, respectively (figure 9)[67]. More recent work showed higher detection limit, below 5 x 10^5 CFU/mL and above 10^6 CFU/mL, by direct loading on PZT material electrode (*i.e.* without any modification of the bacteria) [68].

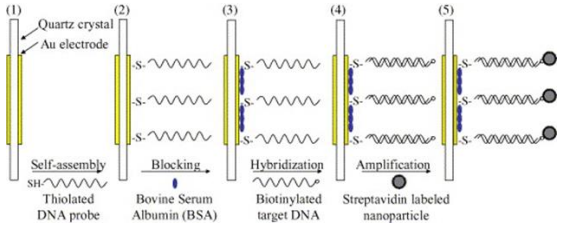


Fig. 9. Quartz crystal microbalance (QCM) sensor fabrication, and procedure for *E. coli* O157:H7 detection. From Mao *et al* [67], reprinted with permission.

E. Carbon nanotubes based biosensors

Carbon nanotubes (CNTs) are considered interesting materials for biosensing applications because these nano-objects can be functionalized with antibodies or aptamers for the detection of bacterial species. They can be synthesized as single-walled carbon nanotubes (SWNTs) composed of a single layer of carbon atoms, with very high aspect ratios (diameters of the order of 1 nm). As an example, Andrade *et al*. [69] propose a new approach for the development of nanostructured biosensors based on carbon nanotubes (CNTs) and antimicrobial peptides for the detection of bacteria (figure 10). The gold electrodes were coated with a self-assembled monolayer of cysteine. The terminal carboxyl groups of CNTs activated by EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide /N-hydroxysuccinimide) allowed the covalent immobilization of CNTs on the surface, and then, of the antimicrobial peptide clavanine A (ClavA) on the NTCs. The surface-immobilized ClavA was then used to detect different concentrations of bacterial cells by electrochemical impedance spectroscopy (EIS) measurements. The infectious agents tested were E. coli, Klesiella pneumoniae, Enterococcus faecalis, and Bacillus subtilis. This biosensor was capable of distinguishing bacterial concentrations in the range of 10^2-10^6 CFU /mL for E. coli, and the authors showed the ability of the sensor to differentiate Gram-negative bacteria (E. coli and K. pneumoniae) from Gram-positive bacteria thanks to electrostatic and hydrophobic interactions with ClavA.

In addition, carbon nanotube (CNT)-based field-effect transistors (FETs) are recent sensing device configurations. When the biological material is adsorbed on the surface of CNTs, electron transfer occurs, which changes their electrical conductivity.

This property has been used for biosensors by functionalizing single valley carbon nanotube based FETs (SWCNT-FETs) with aptamer for E. coli detection by electrical measurements [70]. In this case, the SWNT-FETs showed a decrease of the electrical conductance of more than 50% after binding with 10^7 CFU/mL of *E. coli* to the aptamer-functionalized SWNT-FET. Other work reported [71] the use of SWCNT-FET functionalized with a solution

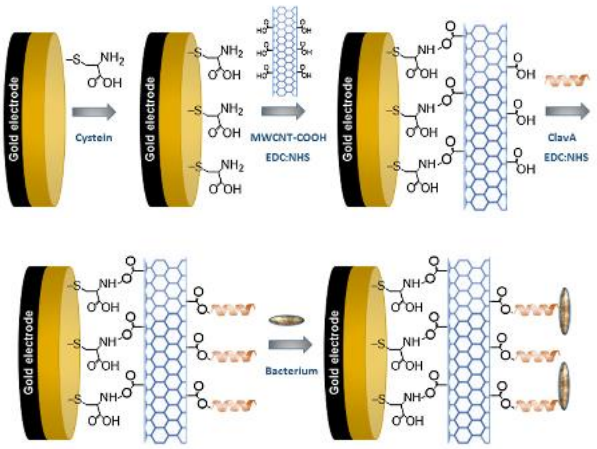


Fig. 10. Schematic representation of the nanostructured sensor based on carbon nanotubes: the peptide is bound on the CNTs surface and the interaction of the sensor with the bacteria is evaluated. From Andrade *et al.* [69], reprinted with permission.

of PASE (1-pyrenebutanoic acid succinimidyl ester) as linker molecule and anti-E-coli antibodies (figure 11). The changes of the electrical resistance of the CNTFET indicate the possibility of this device as biosensor to detect E. coli O_{157}:H_7, and concentration as low as 8.2×10^2 CFU/mL was detected.

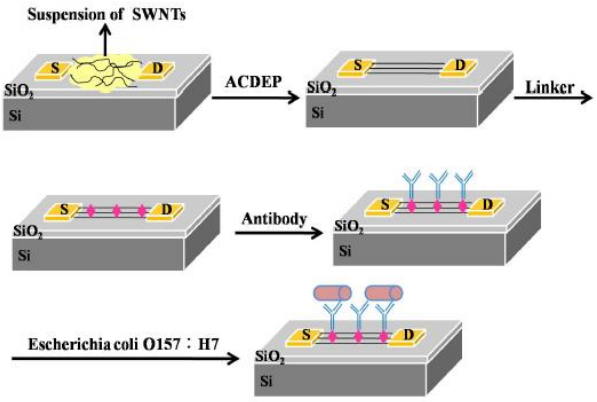


Fig. 11. Schematic illustration for fabrication of CNT-FET E-coli biosensor. From Zhang *et al.* [71], reprinted with permission.

F. Graphene based biosensors

Graphene is a remarkable two-dimensional (2D) material that is of great interest due to its exceptional electrical and thermal conductivity and good mechanical behaviour, with promising applications in electronic devices, especially sensors. The most common methods of graphene synthesis include chemical vapour deposition (CVD) and mechanical exfoliation. Graphene, which is a single atom-thick flat sheet of carbon atoms perfectly arranged in a honeycomb network, has great potential for biosensing. Huang *et al.* have demonstrated a graphene-based biosensor for electrically detecting *E. coli* with high sensitivity and specificity [72]. To specifically detect *E. coli*, anti-*E. coli* antibodies were first immobilized on the graphene film *via* 1-pyrene-butanoic acid succinimidyl ester linked with graphene by π–π stacking. A significant increase in the conductance of the graphene device was observed after exposure to *E. coli* K12 at a concentration as low as 10 CFU/mL, while no significant response was triggered by a high concentration of another bacterial strain. In addition, graphene-based FET devices functionalized with antibodies or antimicrobial peptides (AMPs) have been used for electronic detection of *E. coli*. Thakur *et al.* developed a field effect transistor based on thermally reduced graphene oxide (rGO FET) passivated with an ultrathin layer of Al_2O_3 [73].

Self-assembly of thiol was used to anchor the GO to the interdigital Au electrodes with fingers spaced by 2 µm. To anchor a high amount of anti-*E. coli*, Au nanoparticles (NP) functionalized by glutathione were sprayed onto the FET. The anti-*E. coli* anchored to the AuNP surface selectively captures the *E. coli* cells, and this lead to a change in the

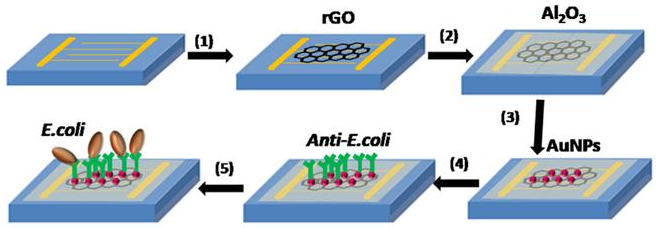


Fig. 12. Schematic depicting various steps involved in the fabrication of FET sensor (1) Self-assembly of rGO (2) deposition of atomic layer of Al2O3 (3) deposition of AuNPs (4) immobilization of anti-*E. coli* and (5) *E. coli* sensing From Thakur *et al.* [73], reprinted with permission.

electrical conductivity of the rGO channel. The electrical conductivity of the sensor was recorded by monitoring the change in drain current for different concentrations of *E. coli* solutions, since the transistor is back-gate biased. The high negative charge density on the surface of *E. coli* cells strongly modulated the concentration of the majority charge carriers in the monolayer, allowing real-time monitoring of the *E. coli* concentration in a given sample. With a low single cell detection limit, the FET sensor had a linear range of 1 to 100 CFU in 1 µL sample volume (i.e. 10^3 to 10^5 CFU/mL) [73] (figure 12).

Techniques have also been used to produce graphene nanoribbons less than 10 nm wide, which are of interest due to quantum confinement of electrons and edge effects. As an example, Chen *et al.* highlighted a new graphene derivative, the holey reduced graphene oxide (hRGO), functionalized with positively charged Magainin I to link Gram-negative bacteria by electrostatic interaction [74]. Even after functionalization, this material preserved its electronic properties. Fan *et al.* demonstrated a graphene-based biosensor to electrically detect *E. coli* with a high sensitivity and specificity [75].

TABLE I
SUMMARY OF RECENT BIOSENSORS BASED ON NANO-OBJECTS FOR *E. COLI* DETECTION

Nano-material	Bacteria	Detection technique	Range concentration (CFU/mL)[a]	Detection limit (CFU/mL)[a]	Characteristics
ZnO nanorods array	*E. coli* O 157:H7 (DNA)	Light-addressable potentiometry	10 - 10^5	10^2	Label-free, sensitive and effective detection of bacterial DNA [40]
Cysteine modified gold NPs	*E. coli* ATCC 25922	Cyclic voltammetry and impedance spectroscopy	1- 10^4	-	Viable, simple and specific detection [45]
Polypyrrole-reduced graphene oxide modified gold nanoparticles	*E. coli* K12	Pulse difference voltammetry	10 - 10^7	10	Enzyme free, good stability, high selectivity and excellent reproducibility detection [50]
ZnONWS/AuNPs/QD-Cds composites	*E. coli* O 157:H7	Photo-electrochemical (amperometry)	10 - 10^7	1.125	Low noise, fast, and ultrasensitive detection [51]
Silver nanoparticles	*E. coli* ATCC 25922	Surface plasmon resonance	1.5×10^1 – 1.5×10^6	0.576	Real-time, label-free, selective, and low detection limit [54]
Graphene quantum dots/SilverNPs nanocomposites	*E. coli*	Electrogenerated chemiluminescence	10 - 10^7	5	Low-cost, real-time, smartphone based detection [61]
Carbon quantum dots/magnetic nanoparticles(Fe_3O_4)	*E. coli* 0 157 :H7	Fluorescence	500 - 10^6	487	Sensitive, specific detection with low-cost, simple method for preparation of nanocomposites [64]
Single walley carbon nanotubes	*E. coli*	Impedance spectroscopy	10^2 - 10^6	10^2	Differentiation of gram-negative and gram-positive bacteria, good reproducibility, very sensitive [65]
Single walley carbon nanotubes	*E. coli* O157:H7	Conductance measurements	8.2×10^2 – 8.2×10^8	-	Real-time detection, and compatible with electronic platform [71]
Graphene	*E. coli* K12	Conductance measurements	10 - 10^5	-	Real-time detection, and compatible with electronic platform [72]
Graphene	*E. coli* ATCC 25922	Conductance measurements	10^3 - 10^5	-	Real-time detection, and compatible with electronic platform [73]
Graphene	*E. coli* O78:K80:H11	Cyclic voltammetry and impedance spectroscopy	10 -10^6	-	Real-time, sensitive and specific detection [76]
Silicon nanowires array	*E. coli* ATCC 35218	Conductance measurements	8×10^3 - 8×10^8	-	Real-time detection, and compatible with CMOS electronic platform [76]

Kaur *et al.* used bacterial-DNA interactions using a nanostructured electrode-based sensor, using CNTs in transverse and longitudinal directions and the subsequent bridging with terephthalaldehyde (TPA), to form 3D bridged rebar graphene. This material exhibited enhanced electrical properties and facilitated chemical functionality. It was functionalized with a specific anti-*E. coli* DNA aptamer. The developed nanostructured aptasensor demonstrated a low detection limit and a sensitivity of ~ 10^1 CFU/mL towards *E. coli* [76].

G. Silicon nanowire-based biosensors

Silicon nanowires (SiNW) are also potential functional probes for pathogen detection due to their unique physical properties. Their one-dimensional structure provides the smallest confinement for electron transport in the longitudinal direction, their large surface area promotes interaction between target cells and nanomaterials, thus leading to a higher sensitivity. They offer unique advantages for the development of pathogen sensors in several respects. The advantages of this smaller scale approach are the possibility of mass production and reduced unit costs thanks to recent advances in micro and nano-manufacturing technologies compatible with CMOS (Complementary Metal Oxide Semiconductor) silicon technology. The lab-on-a-chip system for the rapid detection of bacteria could revolutionize health prevention. Some work has been reported on the use of SiNWs as sensitive units for chemical and biochemical species, but few works reported on the relevance of using the SiNW network, in particular for bacteria detection. The interest of sensors using SiNWs as sensitive units is based on a large contact surface (high surface/volume ratio for each nanowire) allowing potential significant interactions with bacteria, inducing a change in electrical conduction through the network of silicon nanowires after their binding. Indeed, when the device with functionalized nanowires is exposed to macromolecules with a net positive or negative electrical charge, this leads to a change in the charge density at the surface of the nanowire, and thus a change of the global

electrical resistance. In particular, Le Borgne *et al* [77] demonstrated E. coli ATCC 35218 E. coli detection in the range 8×10^3-8×10^8 CFU/mL using SiNWs array based electrical resistor. Some other works showed that nanostructures promote adhesion of cells including bacteria. In particular, Wang *et al*. [78] reported that the graft density

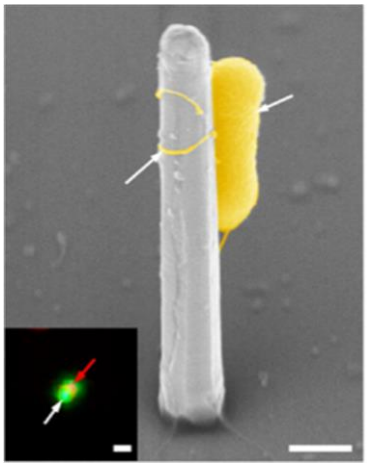


Fig. 13. SEM images showing bacteria attached on the Si patterned nanowires and its corresponding merged image of fluorescence micrographs Si nanowire. From Jeong *et al*. [79], reprinted with permission.

on the modified nanowire arrays was much higher than on analogous smooth silicon, leading to higher bacterial adhesion on the nanowire arrays. Moreover, some studies have shown that nanostructures and their specific physico-chemical properties can be used to regulate the responses and movements of bacterial cells. Jeong *et al*. [79], used a well-defined silicon nanowire array platform and single cell imaging, and showed that the attachment locations of bacteria are strongly influenced by the presence of nanowires on a surface, with preferential attachment to nanowires (figure 13). These results demonstrated that nanoscale topography could affect movement and attachment of bacteria. According to Kim *et al*.[80], the coupling between microcellular species and semiconductor nanomaterials in a cellular nanotransistor enables the charge carriers to be confined to one or two dimensions and to the bio/nano interface, which leads to a very sensitive modification of the density of the carriers. In other work, Susarrey-Arce *et al*. [81] investigated the interaction and the viability of bacteria on SiNWs surfaces, enhanced with different chemical functionalities, using model microorganisms E. coli and S. aureus. Recently, Li *et al* [82] have shown fast bacterial identification in urine samples by capturing bacteria on a microchannel silicon nanowire microfluidic chip, followed by matrix-assisted laser desorption ionization-time of flight mass spectroscopy (MALDI-TOF MS) detection. Bacteria with a concentration of 10^6 CFU/mL in urine samples have been detected.

IV. Conclusion

Rapid and sensitive detection of pathogenic bacteria is extremely important for medical diagnosis or for monitoring pathogens in various food products. The limitations of most conventional diagnostic methods are a lack of ultra-sensitivity or a delay in obtaining results. This review draws a state of the art of biosensors based on nano-objects measuring the presence or activity of *E. coli*, highlighting the technological challenges in terms of sensitivity, specificity, reproducibility, and the limitations and advantages of simplicity and transportability (Table 1). Detection becomes more sensitive and more flexible using nanoparticles as markers, and real-time electrical detection methods are dominant in comparison with optical ones. The lowest detection limit can be achieved for sensors based on metal with optical detection techniques in contrast with electrical detection methods (and more specifically using measurement conductance). Biosensors based on nano-objects for *E-coli* detection are of particular interest for label-free, real-time, ultra-high sensitive, and required selective detection to effectively counteract food-borne infections for example. Further improvements are needed for the development of such sensors because of their potential integration in large-scale addressable network compatible with the fabrication of biological lab-on-chip, that distinguish them from other sensor technologies available today.

References

[1] S. E. Majowicz et al., « Global incidence of human Shiga toxin–producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis », Foodborne pathogens and disease, vol. 11, no 6, p. 447-455, 2014.

[2] B. Biswas, P. Basu, et M. Pal, « Gram staining and its molecular mechanism », in International review of cytology, vol. 29, Elsevier, 1970, p. 1-27.

[3] J. Humbert, M. Jouve, C. Le Bouguénec, et P. Gounon, « Electron microscopic improvement in the study of diarrheagenic Escherichia coli », Microscopy research and technique, vol. 49, no 4, p. 383-393, 2000.

[4] H. Sträuber et S. Müller, « Viability states of bacteria—specific mechanisms of selected probes », Cytometry Part A, vol. 77, no 7, p. 623-634, 2010.

[5] C. L. Marolda, P. Lahiry, E. Vinés, S. Saldías, et M. A. Valvano, « Micromethods for the characterization of lipid A-core and O-antigen lipopolysaccharide », in Glycobiology Protocols, Springer, 2006, p. 237-252.

[6] A. C. Paletta, V. S. Castro, et C. A. Conte-Junior, « Shiga Toxin-Producing and Enteroaggregative Escherichia coli in Animal, Foods, and Humans: Pathogenicity Mechanisms, Detection Methods, and Epidemiology », Current Microbiology, p. 1-9, 2019.

[7] M. Jouve, M.-I. Garcia, P. Courcoux, A. Labigne, P. Gounon, et C. Le Bouguénec, « Adhesion to and invasion of HeLa cells by pathogenic Escherichia coli carrying the afa-3 gene cluster are mediated by the AfaE and AfaD proteins, respectively. », Infection and Immunity, vol. 65, no 10, p. 4082-4089, 1997.

[8] G. Zhou et al., « ASCA, ANCA, ALCA and many more: are they useful in the diagnosis of inflammatory bowel disease? », Digestive Diseases, vol. 34, no 1-2, p. 90-97, 2016.

[9] E. De Boer et A. Heuvelink, « Methods for the detection and isolation of Shiga toxin-producing Escherichia coli », Journal of applied microbiology, vol. 88, no S1, p. 133S-143S, 2000.

[10] H. Chart et C. Jenkins, « The serodiagnosis of infections caused by Verocytotoxin-producing Escherichia coli », Journal of applied microbiology, vol. 86, no 5, p. 731-740, 1999.

[11] W. M. Dunne Jr, H. Pouseele, S. Monecke, R. Ehricht, et A. van Belkum, « Epidemiology of transmissible diseases: array hybridization and next generation sequencing as universal nucleic acid-mediated typing tools », Infection, Genetics and Evolution, vol. 63, p. 332-345, 2018.

[12] N. Jahandeh, R. Ranjbar, P. Behzadi, et E. Behzadi, « Uropathogenic Escherichia coli virulence genes: invaluable approaches for designing

DNA microarray probes », Central European journal of urology, vol. 68, no 4, p. 452, 2015.

[13] I. M. Mackay, K. E. Arden, et A. Nitsche, « Real-time PCR in virology », Nucleic Acids Research, vol. 30, no 6, p. 1292-1305, 2002, doi: 10.1093/nar/30.6.1292.

[14] A. Salman, H. Carney, S. Bateson, et Z. Ali, « Shunting microfluidic PCR device for rapid bacterial detection », Talanta, vol. 207, p. 120303, 2020.

[15] A. Ahmed, J. V. Rushworth, N. A. Hirst, et P. A. Millner, « Biosensors for whole-cell bacterial detection », Clinical microbiology reviews, vol. 27, no 3, p. 631-646, 2014.

[16] N. Gadsby et al., « Development of two real-time multiplex PCR assays for the detection and quantification of eight key bacterial pathogens in lower respiratory tract infections », Clinical microbiology and infection, vol. 21, no 8, p. 788-e1, 2015.

[17] S. Kaushik, U. Tiwari, Nilima, S. Prashar, B. Das, et R. K. Sinha, « Label-free detection of E scherichia coli bacteria by cascaded chirped long period gratings immunosensor », *Rev. Sci. Instrum.*, vol. 90, nº 2, p. 025003, 2019.

[18] Y. Song *et al.*, « Recent progress in microfluidics-based biosensing », *Analytical chemistry*, vol. 91, nº 1, p. 388-404, 2018.

[19] S. M. Yoo et S. Y. Lee, « Optical biosensors for the detection of pathogenic microorganisms », Trends in biotechnology, vol. 34, no 1, p. 7-25, 2016.

[20] H. Chen, Q. Qiu, S. Sharif, S. Ying, Y. Wang, et Y. Ying, « Solution-phase synthesis of platinum nanoparticle-decorated metal-organic framework hybrid nanomaterials as biomimetic nanoenzymes for biosensing applications », ACS applied materials & interfaces, vol. 10, no 28, p. 24108-24115, 2018.

[21] X. Zhang et al., « Recent progress on the construction of nanozymes-based biosensors and their applications to food safety assay », TrAC Trends in Analytical Chemistry, p. 115668, 2019.

[22] J. Ji, J. A. Schanzle, et M. B. Tabacco, « Real-time detection of bacterial contamination in dynamic aqueous environments using optical sensors », Analytical chemistry, vol. 76, no 5, p. 1411-1418, 2004.

[23] C.-C. You et al., « Detection and identification of proteins using nanoparticle–fluorescent polymer 'chemical nose'sensors », Nature nanotechnology, vol. 2, no 5, p. 318, 2007.

[24] R. L. Phillips, O. R. Miranda, C.-C. You, V. M. Rotello, et U. H. Bunz, « Rapid and efficient identification of bacteria using gold-nanoparticle–poly (para-phenyleneethynylene) constructs », Angewandte Chemie International Edition, vol. 47, no 14, p. 2590-2594, 2008.

[25] J. G. Bruno, K. Francis, M. Ikanovic, P. Rao, S. Dwarakanath, et W. E. Rudzinski, « Reovirus detection using immunomagnetic-fluorescent nanoparticle sandwich assays », Journal of Bionanoscience, vol. 1, no 2, p. 84-89, 2007.

[26] A. Bajaj et al., « Detection and differentiation of normal, cancerous, and metastatic cells using nanoparticle-polymer sensor arrays », Proceedings of the National Academy of Sciences, vol. 106, no 27, p. 10912-10916, 2009.

[27] J. Gao, Y. Lai, C. Wu, et Y. Zhao, « Exploring and exploiting the synergy of non-covalent interactions on the surface of gold nanoparticles for fluorescent turn-on sensing of bacterial lipopolysaccharide », Nanoscale, vol. 5, no 17, p. 8242-8248, 2013.

[28] K. Zagorovsky et W. C. Chan, « A plasmonic DNAzyme strategy for point-of-care genetic detection of infectious pathogens », Angewandte Chemie International Edition, vol. 52, no 11, p. 3168-3171, 2013.

[29] Y.-C. Chang, C.-Y. Yang, R.-L. Sun, Y.-F. Cheng, W.-C. Kao, et P.-C. Yang, « Rapid single cell detection of Staphylococcus aureus by aptamer-conjugated gold nanoparticles », Scientific reports, vol. 3, no 1, p. 1-7, 2013.

[30] S. Díaz-Amaya, L.-K. Lin, A. J. Deering, et L. A. Stanciu, « Aptamer-based SERS biosensor for whole cell analytical detection of E. coli O157: H7 », *Analytica chimica acta*, vol. 1081, p. 146-156, 2019.

[31] A. D. Taylor, Q. Yu, S. Chen, J. Homola, et S. Jiang, « Comparison of E. coli O157: H7 preparation methods used for detection with surface plasmon resonance sensor », Sensors and Actuators B: Chemical, vol. 107, no 1, p. 202-208, 2005.

[32] S.-H. Ohk et A. K. Bhunia, « Multiplex fiber optic biosensor for detection of Listeria monocytogenes, Escherichia coli O157: H7 and Salmonella enterica from ready-to-eat meat samples », Food microbiology, vol. 33, no 2, p. 166-171, 2013.

[33] J. Riu et B. Giussani, « Electrochemical biosensors for the detection of pathogenic bacteria in food », TrAC Trends in Analytical Chemistry, p. 115863, 2020.

[34] M. Xu, R. Wang, et Y. Li, « Electrochemical biosensors for rapid detection of Escherichia coli O157: H7 », Talanta, vol. 162, p. 511-522, 2017.

[35] N. Dhull et al., « Label-free amperometric biosensor for Escherichia coli O157: H7 detection », Applied Surface Science, vol. 495, p. 143548, 2019.

[36] S. Noh, Y. Choe, V. Tamilavan, M. H. Hyun, H. Y. Kang, et H. Yang, « Facile electrochemical detection of Escherichia coli using redox cycling of the product generated by the intracellular β-d-galactosidase », Sensors and Actuators B: Chemical, vol. 209, p. 951-956, 2015.

[37] S. Noh, Y. Choe, V. Tamilavan, M. H. Hyun, H. Y. Kang, et H. Yang, « Facile electrochemical detection of Escherichia coli using redox cycling of the product generated by the intracellular β-d-galactosidase », Sensors and Actuators B: Chemical, vol. 209, p. 951-956, 2015.

[38] Y. Li et al., « Amperometric immunosensor for the detection of Escherichia coli O157: H7 in food specimens », Analytical biochemistry, vol. 421, no 1, p. 227-233, 2012.

[39] G. A. Zelada-Guillén, S. V. Bhosale, J. Riu, et F. X. Rius, « Real-time potentiometric detection of bacteria in complex samples », Analytical chemistry, vol. 82, no 22, p. 9254-9260, 2010.

[40] Y. Tian et al., « Label-Free Detection of E. coli O157: H7 DNA Using Light-Addressable Potentiometric Sensors with Highly Oriented ZnO Nanorod Arrays », Sensors, vol. 19, no 24, p. 5473, 2019.

[41] P. M. Shaibani et al., « Portable nanofiber-light addressable potentiometric sensor for rapid Escherichia coli detection in orange juice », ACS sensors, vol. 3, no 4, p. 815-822, 2018.

[42] B.-Y. Chang et S.-M. Park, « Electrochemical impedance spectroscopy », Annual Review of Analytical Chemistry, vol. 3, p. 207-229, 2010.

[43] K. Krupinska, P. Geladi, I. Vikholm-Lundin, et B. Lindholm-Sethson, « Detection of low levels of Escherichia coli by electrochemical impedance spectroscopy and singular value decomposition », Chemometrics and Intelligent Laboratory Systems, vol. 163, p. 49-54, 2017.

[44] P. Geng et al., « Self-assembled monolayers-based immunosensor for detection of Escherichia coli using electrochemical impedance spectroscopy », Electrochimica Acta, vol. 53, no 14, p. 4663-4668, 2008.

[45] J. L. de Miranda, M. D. Oliveira, I. S. Oliveira, I. A. Frias, O. L. Franco, et C. A. Andrade, « A simple nanostructured biosensor based on clavanin A antimicrobial peptide for gram-negative bacteria detection », Biochemical Engineering Journal, vol. 124, p. 108-114, 2017.

[46] N. Idil, M. Hedström, A. Denizli, et B. Mattiasson, « Whole cell based microcontact imprinted capacitive biosensor for the detection of Escherichia coli », *Biosens. Bioelectron.*, vol. 87, p. 807-815, 2017

[47] M. Auffan, J. Rose, J.-Y. Bottero, G. V. Lowry, J.-P. Jolivet, et M. R. Wiesner, « Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective », Nature Nanotech, vol. 4, no 10, p. 634-641, oct. 2009, doi: 10.1038/nnano.2009.242.

[48] N. Sanvicens, C. Pastells, N. Pascual, et M.-P. Marco, « Nanoparticle-based biosensors for detection of pathogenic bacteria », TrAC Trends in Analytical Chemistry, vol. 28, no 11, p. 1243-1252, déc. 2009, doi: 10.1016/j.trac.2009.08.002.

[49] J. Wang, « Nanomaterial-based amplified transduction of biomolecular interactions », small, vol. 1, no 11, p. 1036-1043, 2005.

[50] Y. Zou, J. Liang, Z. She, et H.-B. Kraatz, « Gold nanoparticles-based multifunctional nanoconjugates for highly sensitive and enzyme-free detection of E.coli K12 », Talanta, vol. 193, p. 15-22, févr. 2019, doi: 10.1016/j.talanta.2018.09.068.

[51] X. Dong et al., « CdS quantum dots/Au nanoparticles/ZnO nanowire array for self-powered photoelectrochemical detection of Escherichia coli O157:H7 », Biosensors and Bioelectronics, vol. 149, p. 111843, févr. 2020, doi: 10.1016/j.bios.2019.111843.

[52] Y.-C. Chen, K.-H. Yang, C.-Y. Huang, Z.-J. Wu, et Y.-K. Hsu, « Overall photoelectrochemical water splitting at low applied potential over ZnO quantum dots/nanorods homojunction », Chemical Engineering Journal, vol. 368, p. 746-753, juill. 2019, doi: 10.1016/j.cej.2019.03.024.

[53] H.-A. Joung et al., « High sensitivity detection of 16s rRNA using peptide nucleic acid probes and a surface plasmon resonance biosensor », Analytica Chimica Acta, vol. 630, no 2, p. 168-173, déc. 2008, doi: 10.1016/j.aca.2008.10.001.

[54] E. Özgür, A. A. Topçu, E. Yılmaz, et A. Denizli, « Surface plasmon resonance based biomimetic sensor for urinary tract infections »,

Talanta, vol. 212, p. 120778, mai 2020, doi: 10.1016/j.talanta.2020.120778.

[55] X. Michalet, « Quantum Dots for Live Cells, in Vivo Imaging, and Diagnostics », Science, vol. 307, no 5709, p. 538-544, janv. 2005, doi: 10.1126/science.1104274.

[56] S. Ahmadian-Fard-Fini, M. Salavati-Niasari, et D. Ghanbari, « Hydrothermal green synthesis of magnetic Fe3O4-carbon dots by lemon and grape fruit extracts and as a photoluminescence sensor for detecting of E. coli bacteria », Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 203, p. 481-493, oct. 2018, doi: 10.1016/j.saa.2018.06.021.

[57] R. Edgar et al., « High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes », Proceedings of the National Academy of Sciences, vol. 103, no 13, p. 4841-4845, mars 2006, doi: 10.1073/pnas.0601211103.

[58] S. J. Mechery, X. J. Zhao, L. Wang, L. R. Hilliard, A. Munteanu, et W. Tan, « Using Bioconjugated Nanoparticles To MonitorE. coli in a Flow Channel », Chem. Asian J., vol. 1, no 3, p. 384-390, sept. 2006, doi: 10.1002/asia.200600009.

[59] R. Maalouf, W. M. Hassen, C. Fournier-Wirth, J. Coste, et N. Jaffrezic-Renault, « Comparison of two innovatives approaches for bacterial detection: paramagnetic nanoparticles and self-assembled multilayer processes », Microchimica Acta, vol. 163, no 3-4, p. 157-161, 2008.

[60] L. Wang, W. Zhao, M. B. O'Donoghu, et W. Tan, « Fluorescent nanoparticles for multiplexed bacteria monitoring », Bioconjugate chemistry, vol. 18, no 2, p. 297-301, 2007.

[61] S. Li et al., « Electrogenerated chemiluminescence on smartphone with graphene quantum dots nanocomposites for Escherichia Coli detection », Sensors and Actuators B: Chemical, vol. 297, p. 126811, oct. 2019, doi: 10.1016/j.snb.2019.126811.

[62] S. Wang et al., « A homogeneous magnetic bead-based impedance immunosensor for highly sensitive detection of Escherichia coli O157:H7 », Biochemical Engineering Journal, vol. 156, p. 107513, avr. 2020, doi: 10.1016/j.bej.2020.107513.

[63] S.-M. You et al., « Gold Nanoparticle-Coated Starch Magnetic Beads for the Separation, Concentration, and SERS-Based Detection of E. coli O157:H7 », ACS Appl. Mater. Interfaces, vol. 12, no 16, p. 18292-18300, avr. 2020, doi: 10.1021/acsami.0c00418.

[64] X. Hu et al., « Green one-step synthesis of carbon quantum dots from orange peel for fluorescent detection of Escherichia coli in milk », Food Chemistry, vol. 339, p. 127775, mars 2021, doi: 10.1016/j.foodchem.2020.127775.

[65] H. Li et al., « Designing an aptamer based magnetic and upconversion nanoparticles conjugated fluorescence sensor for screening Escherichia coli in food », Food Control, vol. 107, p. 106761, janv. 2020, doi: 10.1016/j.foodcont.2019.106761.

[66] L. Wang, Q. Wei, C. Wu, Z. Hu, J. Ji, et P. Wang, « The Escherichia coli O157:H7 DNA detection on a gold nanoparticle-enhanced piezoelectric biosensor », Sci. Bull., vol. 53, no 8, p. 1175-1184, avr. 2008, doi: 10.1007/s11434-007-0529-x.

[67] X. Mao, L. Yang, X.-L. Su, et Y. Li, « A nanoparticle amplification based quartz crystal microbalance DNA sensor for detection of Escherichia coli O157:H7 », Biosensors and Bioelectronics, vol. 21, no 7, p. 1178-1185, janv. 2006, doi: 10.1016/j.bios.2005.04.021.

[68] J.-Z. Tsai, C.-J. Chen, D.-T. Shie, et J.-T. Liu, « Resonant efficiency improvement design of piezoelectric biosensor for bacteria gravimetric sensing », Bio-Medical Materials and Engineering, vol. 24, no 6, p. 3597-3604, 2014, doi: 10.3233/BME-141186.

[69] C. A. S. Andrade et al., « Nanostructured sensor based on carbon nanotubes and clavanin A for bacterial detection », Colloids and Surfaces B: Biointerfaces, vol. 135, p. 833-839, nov. 2015, doi: 10.1016/j.colsurfb.2015.03.037.

[70] H.-M. So et al., « Detection and Titer Estimation ofEscherichia coli Using Aptamer-Functionalized Single-Walled Carbon-Nanotube Field-Effect Transistors », Small, vol. 4, no 2, p. 197-201, févr. 2008, doi: 10.1002/smll.200700664.

[71] X. Zhang, D. Wang, D. Yang, S. Li, Z. Shen, et others, « Electronic detection of Escherichia coli O157 H7 using single-walled carbon nanotubes field-effect transistor biosensor », Engineering, vol. 4, no 10, p. 94, 2013.

[72] Y. Huang, X. Dong, Y. Liu, L.-J. Li, et P. Chen, « Graphene-based biosensors for detection of bacteria and their metabolic activities », p. 5, 2011.

[73] B. Thakur et al., « Rapid detection of single E. coli bacteria using a graphene-based field-effect transistor device », Biosensors and Bioelectronics, vol. 110, p. 16-22, juill. 2018, doi: 10.1016/j.bios.2018.03.014.

[74] Y. Chen, Z. P. Michael, G. P. Kotchey, Y. Zhao, et A. Star, « Electronic Detection of Bacteria Using Holey Reduced Graphene Oxide », ACS Appl. Mater. Interfaces, vol. 6, no 6, p. 3805-3810, mars 2014, doi: 10.1021/am500364f.

[75] Z. Fan, R. Kanchanapally, et P. C. Ray, « Hybrid graphene oxide based ultrasensitive SERS probe for label-free biosensing », The Journal of Physical Chemistry Letters, vol. 4, no 21, p. 3813-3818, 2013.

[76] H. Kaur, M. Shorie, M. Sharma, A. K. Ganguli, et P. Sabherwal, « Bridged Rebar Graphene functionalized aptasensor for pathogenic E. coli O78:K80:H11 detection », Biosensors and Bioelectronics, vol. 98, p. 486-493, déc. 2017, doi: 10.1016/j.bios.2017.07.004.

[77] B. Le Borgne et al., « Bacteria electrical detection using 3D silicon nanowires based resistor », Sensors and Actuators B: Chemical, vol. 273, p. 1794-1799, 2018.

[78] H. Wang, L. Wang, P. Zhang, L. Yuan, Q. Yu, et H. Chen, « High antibacterial efficiency of pDMAEMA modified silicon nanowire arrays », Colloids and Surfaces B: Biointerfaces, vol. 83, no 2, p. 355-359, 2011.

[79] H. E. Jeong, I. Kim, P. Karam, H.-J. Choi, et P. Yang, « Bacterial recognition of silicon nanowire arrays », Nano letters, vol. 13, no 6, p. 2864-2869, 2013.

[80] S. Kim, B. Keisham, et V. Berry, « Cellular nano-transistor: An electronic-interface between nanoscale semiconductors and biological cells », Materials Today Nano, vol. 9, p. 100063, 2020.

[81] A. Susarrey-Arce et al., « Bacterial viability on chemically modified silicon nanowire arrays », Journal of Materials Chemistry B, vol. 4, no 18, p. 3104-3112, 2016.

[82] Y. Li, T. Wang, et J. Wu, « Capture and detection of urine bacteria using a microchannel silicon nanowire microfluidic chip coupled with MALDI-TOF MS », Analyst, vol. 146, no 4, p. 1151-1156, 2021, doi: 10.1039/D0AN02222E.E.

Yousra BENSERHIR (is PhD student at the University of Rennes (France). She obtained her MSc Nanophysics, Clermont-Auvergne University, France. Her research interest focuses on the development of biosensor for bacteria detection. Her activities include silicon nanowires synthesis, sensors based on silicon nanowires fabrication and electrical measurements.

Anne-Claire SALAÜN is Associate Professor at IETR. Her research activities are focused on silicon nanowires technology for CMOS electronics and sensors applications. She is head manager of the Electrical Engineering and Industrial Computing (EEIC) department. Her research activities are devoted to the polysilicon or silicon nanowires TFT fabrication and micro-technology, with applications to chemical and biological sensors. She focuses on sensors based on silicon nanowires.

Florence GENESTE was graduated from the Ecole Supérieure de Chimie Industrielle de Lyon. She completed her Ph.D. in Chemistry at the University Paris-Sud, where she acquired expertise in Organic Synthesis. She is research director at CNRS in the group "Condensed matter and electroactive systems" of the Institute of chemical sciences of Rennes at the University of Rennes 1. Her research interest is primarily focused on the modification of surfaces for different applications in electrocatalysis, sensors, environment and energy.

Laurent PICHON is professor at the Institut d'Electronique et des Télécommunications de Rennes (IETR) university of Rennes. His research activities are focused on silicon nanowires technology for CMOS electronics and sensors applications. From 2012 to 2016 he managed of the "Microelectronic components" team of the DM2-IETR.

Anne JOLIVET-GOUGEON, M.D., Ph.D. is a graduate of Rennes 1 Medical and Pharmaceutical University. Her topics of research are virulence factors and survival processes in pathogenic oral bacteria, planktonic or in biofilm, antimicrobial susceptibility studies, molecular identification techniques. She is Member of the French Society for Microbiology. She has experience in human Microbiology, as Hospital Practitioner at University hospital of Rennes (medical biology).