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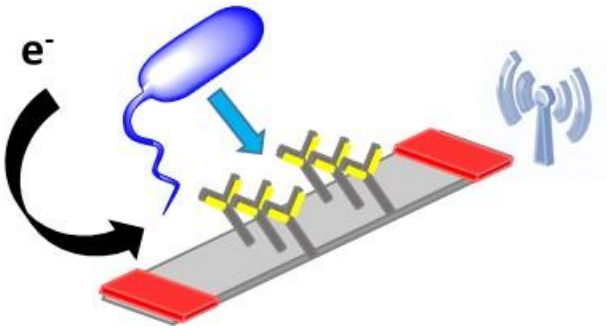

Electrochemical methodologies for the detection of pathogens

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Abstract

Bacterial infections remain of the principal causes of morbidity and mortality worldwide. The number of death due to infections is declining every year by only 1% with a forecast of 13 million deaths in 2050. Among the 1400 recognized human pathogens, the majority of infectious diseases is caused by just a few, about 20 pathogens only. While the development of vaccinations and novel anti-bacterial drugs and treatments are at the forefront of research, and strongly financially supported by policy makers, another manner to limit and control infectious outbreaks is targeting the development and implementation of early warning systems, which indicate qualitatively and quantitatively the presence of a pathogen. As toxin contaminated nutrition and drinks are a potential threat to human health and have consequently a significant socio-economic impact worldwide, the detection of pathogenic bacteria remains not only a big scientific challenge but also a practical problem of enormous significance. Numerous analytical methods, including conventional culturing and staining techniques as well as molecular methods based on polymerase chain reaction amplification and immunological assays have emerged over the years and are used to identify and quantify pathogenic agents. While being highly sensitive in most cases, these approaches are highly time, labor and cost consuming, requiring trained personnel to perform the frequently complex assays. A great challenge in this field is therefore to develop rapid, sensitive, specific, and if possible miniaturized devices to validate the presence of pathogens in cost and time efficient manners.

Electrochemical sensors are well accepted powerful tools for the detection of disease-related biomarkers, environmental and organic hazards. They have also found widespread interest in the

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last years for the detection of waterborne and foodborne pathogens due to their label free character and high sensitivity. This review is focused on the current electrochemical-based microorganism recognition approaches and putting them into context of other sensing devices for pathogens such as culturing the microorganism on agar plate and the polymer chain reaction (PCR) method, able to identifying the DNA of the microorganism. Recent breakthroughs will be highlighted, including the utilization of microfluidic devices and immunomagnetic separation for multiple pathogen analysis in a single device. We will conclude with some perspectives and outlooks to understand better shortcomings. Indeed, there is currently no adequate solution that allows the selective and sensitive binding to a specific microorganism, that is fast in detection and screening, cheap to implement, and able to be conceptualized for a wide range of biologically relevant targets.

Keywords: pathogen; electrochemistry; sensing; toxins; bacteria

Vocabulary section:

1. **Analytical Electrochemistry**: is the application of electrochemical processes to measure the quantity of a species of interest.
2. **Aptamer**s: Nucleic acids that bind to targets with affinities in the micro to picoMolar range, analogous with binding constants of antibodies/antigen interactions
3. **Biosensor**: analytical device involving a biological sensing part with wide range of applications, including food safety, environmental monitoring, and health monitoring.
4. **Biofilm**: biofilms comprise any group of microorganisms in which cells stick to each other and often also to a surface.
5. **Bacterophage**: A virus that infects and replicates within Bacteria

The detection of pathogenic microorganisms remains a big scientific challenge and a practical problem of enormous significance. Per year, an estimated 250 million people are affected by pathogenic bacteria, of which about 8% are fatal.[1] An early state detection of some of these infections would have avoided lengthy treatments and many of the death counts. The gold standard for bacteria detection remains microorganism culturing on agar plates (**Figure 1A**). While bacteria are too small to be visualized with the naked eyes, upon culture on an agar plate with nutrition medium, the bacteria cells can divide rapidly and form a visible patch, appearing as white, cream, or yellow in color, and fairly circular in shape. This diagnostic scheme takes a minimum of 24 hours, ignoring viable but non-culturable cells. Bacteria plating is often followed by standard biochemical identification using a set of tests such as catalase test, citrate test, gram staining, methylene red staining etc. Gram staining using crystal violet/iodine is probably the most commonly used technique as it allows to differentiate Gram positive from Gram negative bacteria. Gram-negative bacteria get decolorized when exposed to alcohol. Gram-negative bacteria can thus subsequently be stained with safranin and appear red in color. This is in contrast to Gram-positive bacteria with a thick peptidoglycan layer with low lipid content. Decolorizing with alcohol causes cell wall dehydration and shrinkage and prevents the release of the stain from the cells and leaving a blue/purple coloring (**Figure 1B**). Another widely applied test is based on the use of methyl red (MR). The principle the MR test is based on the knowledge that some bacteria have the ability to metabolize glucose to pyruvic acid, metabolized to lactic acid, acetic acid or formic acid as end product. The acid produced decreases the pH indicated by a change in the color of MR from yellow to red. For example, *Enterobacter aerogenes* or *Klebsiella pneumonia* give a negative MR test remaining yellow, while *Escherichia coli* ATCC 25922 results in a positive MR test and red color (**Figure 1B**).

To avoid the limitations of culture-dependent techniques, a variety of molecular approaches have been established.[2] The use of molecular techniques allowed the characterization of microbial communities without the requirement of culturing the microorganism. These approaches are based on extraction and purification of bacterial DNA and RNA. Simple fluorescence based approaches[3] using labeled oligonucleotide probes binding to microbial DNA are largely used and are based on is low cytometry (FC), which monitors bacterial abundance and cell viability in suspension using fluorescent dyes (**Figure 1C**).[4]

Next to these approaches, polymer chain reaction (PCR) method introduced in 1980s, is able to identifying the DNA of microorganisms (**Figure 1D**).[5-6] Since 2005, the development of next-generation sequencing, together with decreasing costs for sequencers and reagents have made microbial genomics more accessible. However, while a large number of DNA microarrays are commercially available, not even a handful are adapted for microbial analysis, where low and medium density arrays serve as ideal platforms. Among some of the chips commercially available are *Helicobacter pylori* arrays from MWG Biotech (www.mwg-biotech.com), *M. tuberculosis*, *C. albicans* and *Plasmodium falciparum* from OPERON (www.operon.com), *E. coli* arrays from Pan Vera (www.panvera.com), *E. coli* and *P. aeruginosa* arrays from Affymetrix (www.affymetrix.com), *E. coli* and *M. tuberculosis* arrays from Sigma-Genosys (www.sigma-genosys.com) and others. StaphyChip,[7] developed by Affymetrix, is for example a low-density microarray designed to detect various staphylococcus species. Specifically, it uses PCR with degenerate primers to amplify the *femA* gene, a highly conserved staphylococcal peptidoglycan gene. These DNA products are then analyzed on the microarray bearing capture probes specific to five staphococcal species that are most closely linked to hospitalization-related infections—*S. aureus*, *S. haemolyticus, S. epidermidis*, *S. saprophyticus* and *S. hominis*. A microarray platform to distinguish between different *Pseudomonas* species was developed by researchers at Michigan State University.[8] Unlike the previous example, PCR-amplified genomic fragments were immobilized on the array interface rather than small oligonucleotides and probed the arrays with labeled fragments of genomic DNA from various bacterial isolates.

The advantages of these approaches are the quick profiling, detection of different microorganisms simultaneously, and the possibility to analyze a large number of samples simultaneously. The drawbacks are that all these approaches remain rather time consuming and laborious. In the case of the FISH method, for specific detection the sequence information for probe design is required. The high cost and time-consuming data analysis of high-throughput sequencing techniques as well as microarray technology is often a limiting factor for their application.

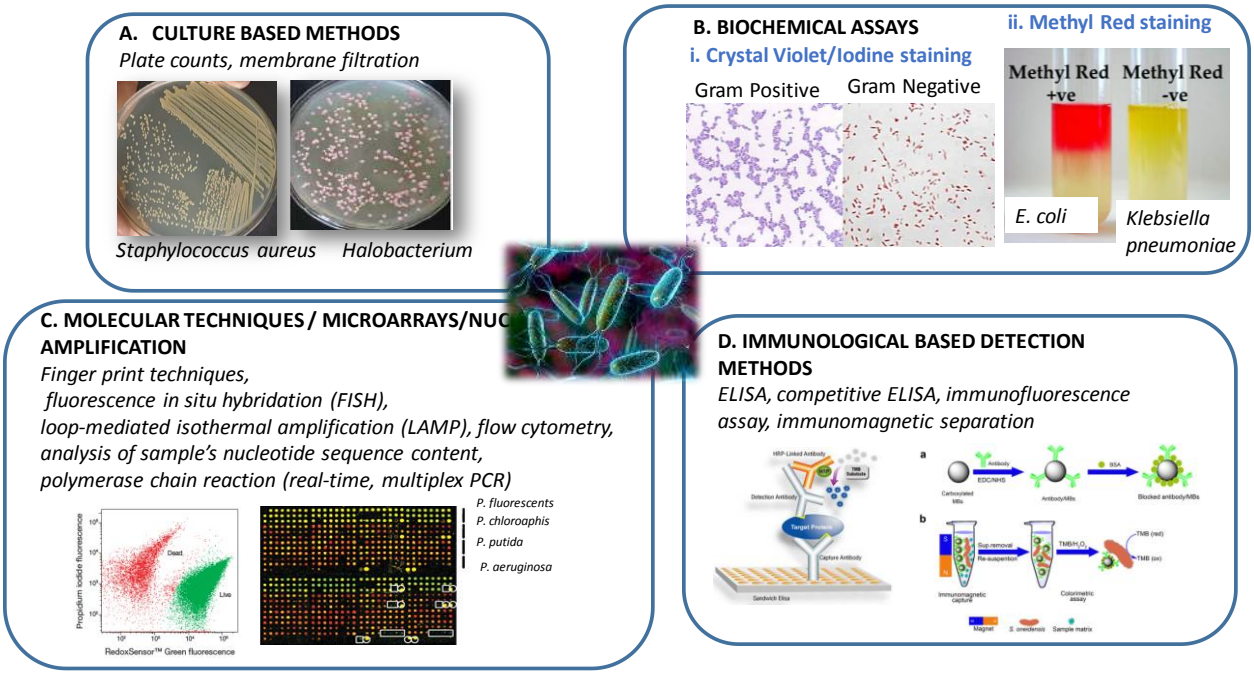


Figure 1: Current approaches for bacteria detection and quantification: (A) Culture-dependent approach such as plate counting; (B) Differentiation between bacteria strains using biochemical assays such as crystal violet/iodine staining or the methylene red assay; (C) Molecular approaches: (left): Flow cytometry image of killed and healthy *S. aureus* cells when stained with propidium iodide and RedoxSensor Green (Reprinted with permission from Ref.[9]; Copyright 2008/Springer Nature,); (right) PCR based methods: image of microarray used for the diagnosis of infections using PCR-amplified genomic fragments from various *Pseudomonas* spp. (Reprint with permission from Ref.[8]; Copyright 2001, American Society for Microbiology); (D) Immunological based methods: (left) sandwich ELISA, (right) Colorimetric immunomagnetic assay.

Apart from the specific sequences that reside in the genome of bacteria, antibodies can be used for specific targeting of bacteria. Immunological-based methods, using polyclonal or monoclonal antibodies, have become common methods for bacteria detection (**Figure 1D**). Although immunological detection methods less sensitive and specific than nucleic-acid based schemes, they are faster and the sensitivity can be enhanced through heterogeneous assays such as enzyme-linked immunosorbent assays (ELISA), competitive ELISA, ELISA coupled to immunomagnetic separation or immunofluorescence assays. Sandwich ELISA is mainly used, where a capture antibody is coated to the solid phase in an ELISA plate. After addition of the bacteria solution, detection antibodies are added. Further exposure to enzyme-linked antibodies

will result in a color change in the presence of a substrate (e.g. 3, 3′, 5, 5′-tetramethylbenzidine (TMB)) in the case of a positive event which can be measured with a spectrophotometer where the intensity is proportional to the level of antibodies present in the samples. In the case of immunofluorescence assays, antibodies are labeled with fluorescence reporter molecules and can be used directly to detect bacteria in clinical samples. It is to be noted that the use of polyclonal antibodies will lack specificity. Immunomagnetic separation involves magnetic nanoparticles coated with antibodies. Adding these beads to bacteria solutions results in bacteria capture, which can be magnetically isolated.[10] Analysis of the captured bacteria is then possible using peroxidase-amplified colorimetric readout.

While in plate based ELISA tests antigen binding to bacteria and its detection stages are separated in time, the use of biosensors have the intrinsic advantage that bacteria binding to antibody directly triggers a signal that can be detected by a proper detector (depending on the design of the detection system (**Figure 2**). The term "biosensor" describes an analytical device involving a biological sensing part with wide range of applications, including food safety, environmental monitoring, and health monitoring. The advantages of biosensors for bacteria detection compared to other molecular analysis techniques are their cost effectiveness and fast response generally within the minute range. When compared to the ELISA format, the use of flow cells and small surface of immersed probe makes ligand-bacteria interaction similar to interaction occurring in a liquid phase. This contrasts with ELISA, where a strictly two-phase system, liquid analyte and solid phase bound antibodies, is used which needs more time to equilibrate between both phases and results in analysis times of hours rather than minutes as is the case of biosensors. While most biosensors cannot currently discriminate between live and dead microorganism, they can give a first fast quantitative and qualitative estimation of the pathogens, which is in most cases enough to decide on the follow up steps.

The use of electrochemical sensors, which started in 1962 with the construction of a glucometer using glucose oxidase-based sensors, has become widely accepted concept. The possibility of miniaturization, multiplexing together with the ability of construction of flexible, disposable and cheap electrochemical sensing devices has made electrochemical sensors very attractive for many applications where sensitivity, simplicity of operation, fast response time, and low cost are

essential. The improvements reported in detection limits as well in specificity of electrochemical biosensors in the last decade is mainly due to the achievements realized in materials science and subsequent availability of a wide range of nanomaterials and composite materials with good electrical conductivity and/or catalytic activity. As most electrochemical and electrical sensors are label-free sensing devices, their potential for pathogen detection is large and have been widely considered as inexpensive and powerful alternatives over molecular pathogen detection approaches. The review focuses on the advancements made in the last 5 years in this field.

First step in the design of an electrochemical pathogen sensor: Surface modification

One important aspect in any biosensor design is linked to the way the pathogen-specific ligand is attached to the surface. A variety of transducer surfaces are available onto which different bioreceptors such as antibodies, polysaccharides, aptamers, bacteriophages, etc can be easily immobilized (**Figure 2**).

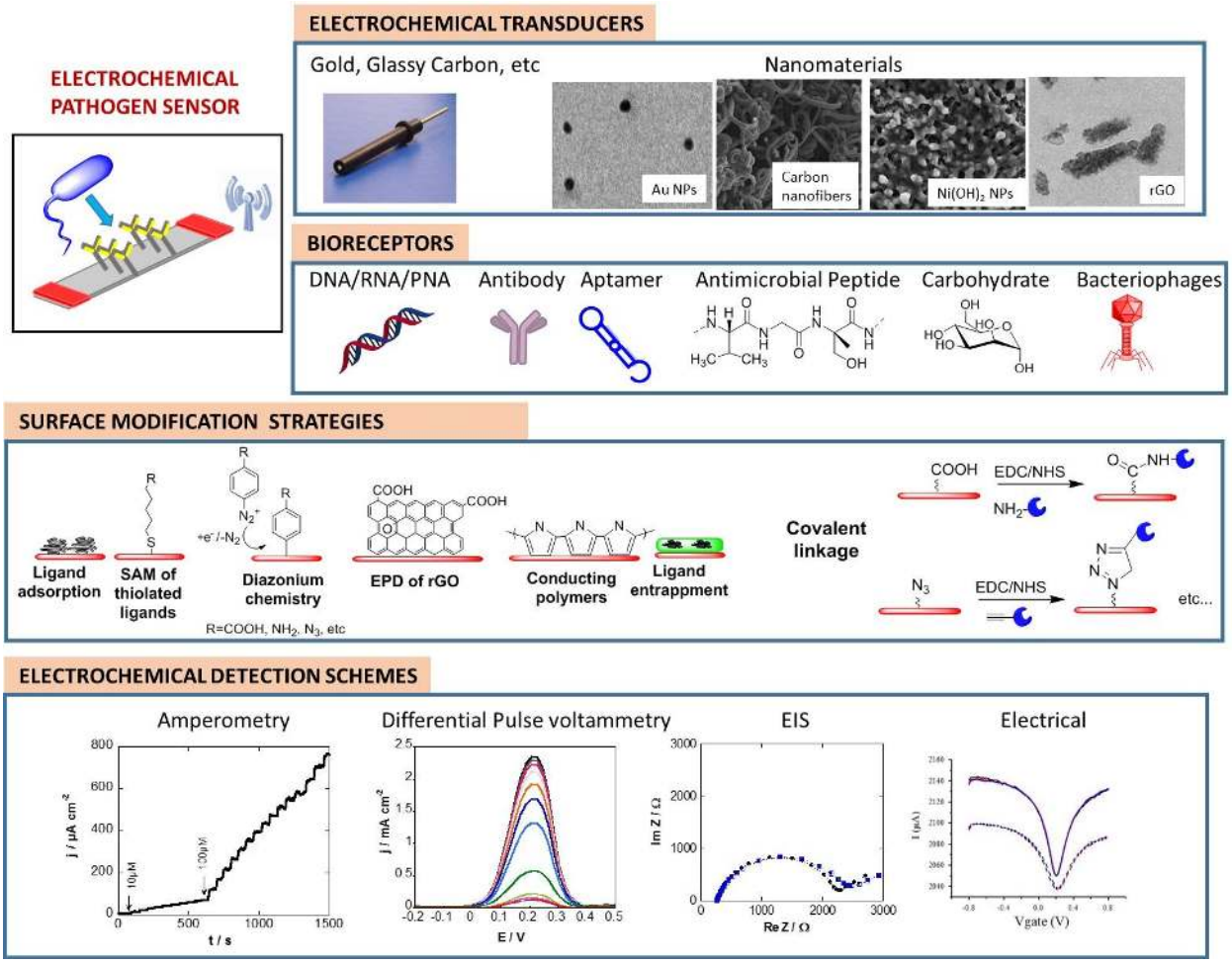


Figure 2: Biosensors for pathogen detection and quantification in food, water and human serum samples: (A) Bioreceptors used; (B) Most common electrode materials used; (C) Surface modification strategies; (D) Different electrochemical detection approaches reported.

Bacteria tend to attach reversibly to solid surfaces during the initial contact; eventually the microorganism starts secreting exopolysaccharides which results in their irreversible adhesion to the surface, often in the form of biofilms.[11] While the timescale in pathogen sensing is usually not enough for biofilms to form, non-specific adsorption has to be minimized to fully take advantage of a biosensing device. Surface chemistry plays thus a significant part not only in linking the biological recognition element onto the sensing surface but also to block the electrode against non-specific interactions. The existing immobilization strategies include bioreceptor adsorption, entrapment and encapsulating in polymers, and covalent linking approaches. Whatever the chosen strategy, it has to be ensured that both the accessibility of the bioreceptor to the target molecule and its recognition capability have to be preserved. The main classes of biological recognition elements used in pathogenic sensors are next to nucleic acids, antibodies, aptamers, baceriophages.[12]

Adsorption

Non-specific adsorption

Adsorption is an easy manner to modify an electrode surface with a pathogen recognition element in a completely random way. Proteins may however partially denature and lose their function. Optimal conditions need to be determined for each biorecognition element, with an optimal surface coverage often being achieved for most proteins on uncharged surfaces at neutral pH and physiological ionic strength using concentrations of 5-20 µg/mL. Yang et al. established an impedimetric immunosensor by adsorption of anti-*E. coli* antibodies onto integrated microelectrode arrays for the detection of *E. coli* O157:H7 with a detection limit being however rather high, 10^6 cfu mL^{-1}.[13]

The surface modification with protein G and A, surface receptors produced by numerous bacteria strains, can promote binding of bioreceptors. As each protein A and G can indeed bind 4 and 2 molecules of IgG.

A similar approach is that using the strong binding of the glycoprotein avidin to biotin-functionalized bioreceptors due to the strong dissociation constant of 1.3×10^{-15} M of the avidin/biotin complex. *E. coli* as low as 10-100 cfu mL^{-1} in concentration could be detected on avidin modified electrodes using biotinylated anti-*E. coli.* as recognition ligand.[14] The high isoelectric point of avidin (IP=10) together with its carbohydrate content made the use of streptavidin, a similar glycan-free protein with an IP of 5-6, of higher interest. However, high levels of non-specific bacteria adhesion were reported on streptavidin chips.[15]

Self-assembled monolayers (SAMs)

SAMs are chemisorbed layers formed by spontaneous organization of thiolated molecules on metallic interfaces, with the most widely used SAMs being derived from *n*-alkanehtiols on gold. [16] Functional SAMs are frequently the base for immobilization of pathogenic recognition elements using covalent approaches. Geng et al. used for example mercaptoacetic acid to from SAM for immobilization of anti-*E. coli* antibodies on gold electrodes with a reported detection limit of 10^3 cfu mL^{-1}.[17] Electrochemical immunosensing platforms composed of thiolated chitosan (CHI–SH) modified electrodes offered also interfaces rich in –SH functional groups.[18]

Covalent attachment

Numerous strategies exist for the covalent coupling of recognition ligands to electrochemical interfaces and lessons from protein arrays were useful in establishing optimal conditions. A commonly used strategy consists in crosslinking carboxylic acid groups on the electrode surface and/or on the biorecognition element with amine groups on ligand or surface, respectively, exploiting amide bond formation using EDC/NHS chemistry. Furthermore, this coupling strategy is also successfully applied to three-dimensional supports, such as agarose, aldehyde–agarose and carboxymethylated dextran modified electrodes.[19]

The use of carboxylic acid modified pyrene ligands is particularly useful for the modification of carbon based materials such as reduced graphene oxide as well as carbon nanotubes through $\pi-\pi$ stacking interaction, offering the possibility for covalent attachment via EDC/NHS chemistry.[20-22] Covalent integration of functional groups using diazonium chemistry has also been lately proposed by some authors.[23-24]

Imprinting

A rather different approach is the entrapment of bacterial recognition ligands or the bacteria itself into polymer and sol-gel matrixes. The dissolution of the imprinted structures results in something which got known as molecular imprinted polymers (MIPs). MIPs are thus nothing else than artificial receptor ligands. As they are resistant to degradation and inactivation, some efforts have been put onto the development of MIPs for electrochemical pathogen detection.[25-30] One of the first MIPs reported by Tothill and co-workers[25] targeted bacteriophage MS2 as template for the detection of water born viruses, using a surface plasmon based read out. Golabi et al. demonstrated recently the potential of cell imprinted polymers as recognition elements for selective bacteria detection.[27] 3-Aminophenylboronic acid (3-APBA) was used for the fabrication of a cell-imprinted polymer (**Figure 3A**). Boronic acid functions are able to specifically and reversibly interact with cis-diol molecules,[31] which facilitates easy release of captured bacterial cells and subsequent regeneration of the CIP. Using *S. epidermidis* as a model target and EIS as a detection method, detection in the range of 10^3-10^7 cfu mL^{-1} was achieved.

Roy et al. reported an impedimetric sensor based on cell-mediated films for *E. coli* using of Ag-ZnO nanoparticle and graphene oxide (Ag-ZnO @GO).[30] Using scanning wave voltammetry and ferrocyanide redox species, the sensor detected *E. coli* as low as 10 cfu mL^{-1} and allowed capturing 98% of the bacterial cells, which could be in addition ablated photothermally. An electrochemiluminescence (ECL) biosensor based on a polydopamine imprinted polymer and nitrogen-doped graphene quantum dots (N-GQDs) for *E. coli* O157:H7 detection was proposed by Chen et al. (**Figure 3B**)[22d]. Removal of *E. coli O157:H7* from the polydopamine film, selective recognition is achieved upon labeling with *E. coli O157:H7* polyclonal antibody (pAb) conjugated to N-GQDs, which generates intensive ECL in the presence of $K_2S_2O_8$. Under optimal conditions a limit of detection of 8 cfu mL^{-1} was attained. Jiang et al. opted for the synthesis of magnetic molecular imprinted polymer on glassy carbon electrodes capable of selectively absorbing Gram-negative bacterial signaling molecules. Using differential pulse voltammetry (DPV), quorum signaling molecules could be detected down to 0.8 nM.[28]

(A)

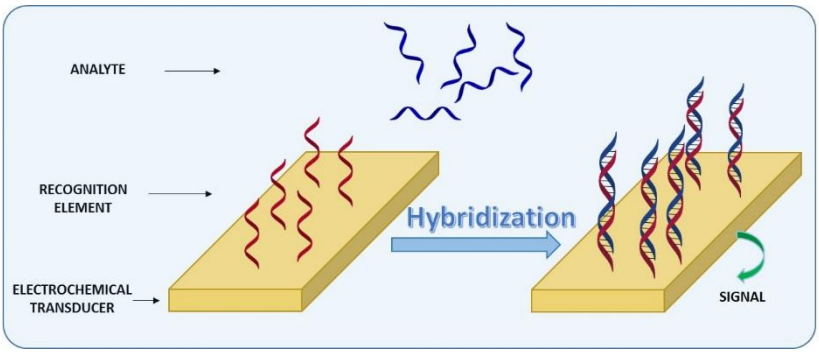
(B)

Figure 3: EIS based whole cell imprinting sensor for *S. epidermis* detection. (A) (a) Imprinting strategy: Boronic acid groups attach to the surface of bacteria, electropolymerisation; Bacterial cells are removed, complementary cavities remain; (b) SEM images of *S. epidermidis* captured in imprinted polymer before and after removal; (c) EIS results upon incubation with different concentrations of *S. epidermidis* together with calibration curve. Experimental details: 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) in PBS; frequency range 0.1–10,000 Hz, bias potential of 0.0 mV *vs.* OCP. (Reprint with permission Ref.[27] Copyright 2017, Elsevier); (B) (a) Illustration of fabrication of biosensor with detection process, (b) ECL trace for different *E. coli* concentrations together with calibration curve (Reprint with permission from Ref.[26]; Copyright 2017/American Chemical Society)

DNA based sensors

DNA sensors have revolutionized modern analysis due to their stability and low-cost, making them a versatile building block for the fabrication of new devices in nanotechnology and biosensor technology. Most literature has focused on genosensors due to their high specificity. Electrochemical DNA sensors are also effective tools for the rapid detection of pathogens with high sensitivity and selectivity. The most crucial step in the preparation is the surface immobilization of the DNA strands. Subsequent interaction with a specific fragment gene from the pathogen causes changes in the DNA structure or the assembly and the electrochemical properties of the interface which can be assessed using impedimetric as well as voltammetric read outs (**Figure 4A**). Electrochemical detection of DNA are divided into direct and indirect approaches (**Figure 4B**). The direct methods use the electroactivity of DNA itself or the changes in the interfacial properties of the DNA-modified electrode such as capacitance, conductivity or impedance. The indirect methods rely on the use of electrochemical active DNA intercalators[32] (e.g. methylene blue) [33-34] or labels[35] such as enzymes (e.g. horseradish peroxidase),[36] redox mediators (e.g. $Ru(NH_3)_6^{2+}$),[37] particles[34] to amplify the read out.[38-40] The literature is rather rich (**Table 1**) and some examples will be highlighted in the following section.

(A)



(B)

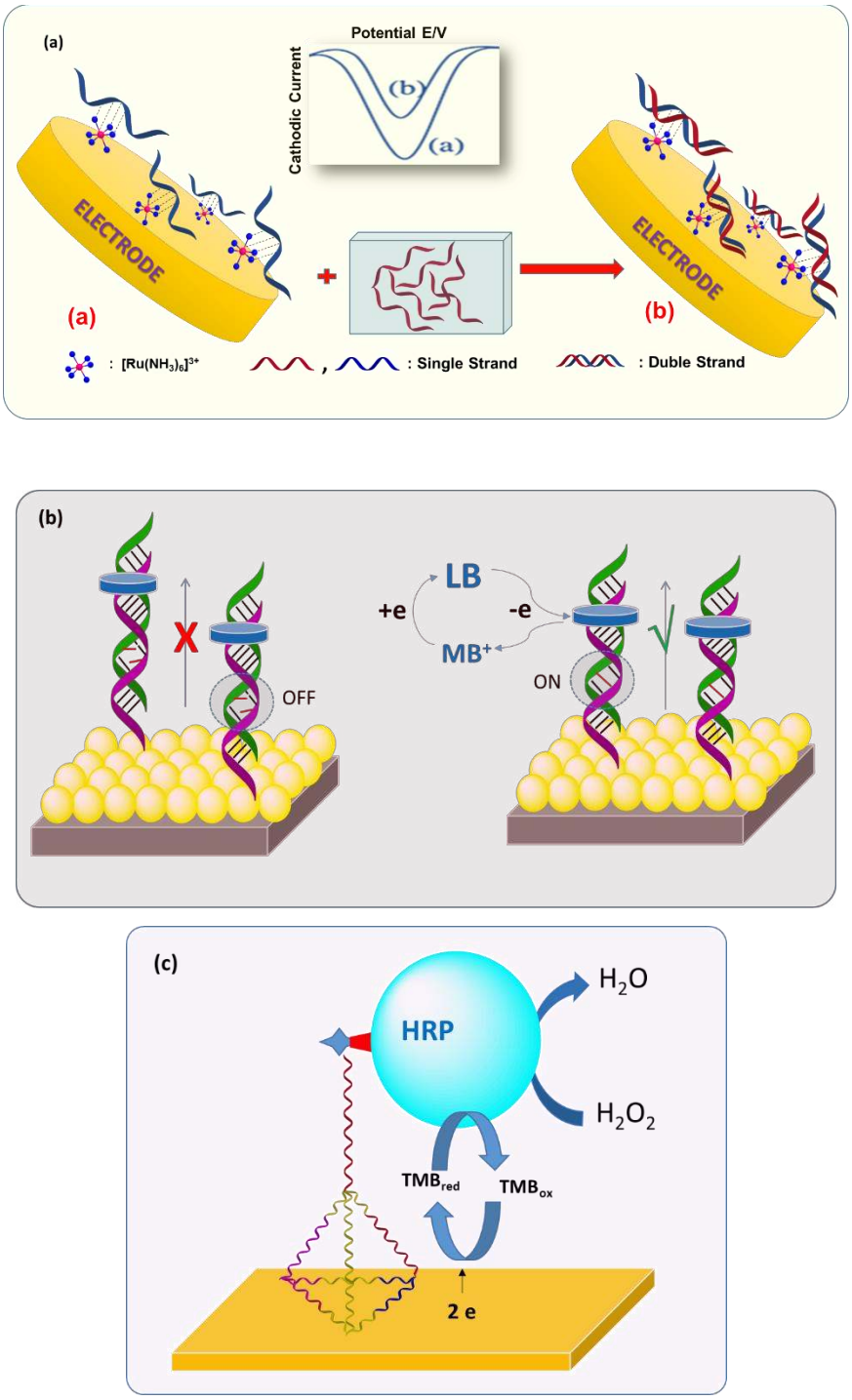


Figure 4. Schematic of a DNA biosensor for pathogen detection: (A) Direct method; (B) Indirect method based on (a) $Ru(NH_3)_6^{2+}$ as redox mediator, (b) methylene blue, (c) enzyme (HRP).

Electrochemical detection of *E. coli* and *S. aureus* using a LAMP amplicon was demonstrated by Ahmed et al.[37] Abdalhai et al. reported an electrochemical genosensor for the detection of pathogenic *E. coli* O157:H7 in fresh beef samples.[38] The genosensor was fabricated by immobilization of DNA molecules on a gold surface, able to hybridize with a specific fragment gene from *E. coli*. Further interaction with CdS NPs modified with sDNA resulted in a sandwich structure, which upon immersion into HNO_3 resulted in the release of Cd^{2+}. The amount of Cd^{2+} detected is proportional to the amount of pathogenic *E. coli* O157:H7.[38]

A sensor consisting of multiwalled carbon nanotubes (MWCNTs) covered with polypyrrole, polyamido amine dendrimers and ferrocenyl groups as a redox marker was proposed by Miodek et al. for electrochemical sensing of rpoB gene of *M. tuberculosis* in real PCR samples with a detection limit of 0.3 fM (**Figure 5A**).[41]

Amperometric DNA sensors were found also to be valuable tools for the detection of viruses.[36] A DNA probe was immobilized onto a gold electrode through self-assembly using thiolated nucleotide sequences and a longer nucleotide sequence of complementary DNA. The captured target sequence was hybridized using biotinylated ssDNA oligonucleotide. Addition of avidin-labeled horseradish peroxidase and 3,3′,5,5′-tetramethylbenzidine as substrate molecule allowed for the specific recognize of target DNA fragments of influenza viruses with a detection limit of 100 fM for target nucleotide sequences.[36]

Vi. parahaemolyticus, a bacterium being the leading cause of seafood-associated gastroenteritis and one of the most important food-borne pathogens, could be sensed electrochemically using screen printed carbon electrodes modified with polylactide-stabilized gold nanoparticles and MB (methylene blue).[34] Detection was assessed through the reduction of peak current of methylene blue against log C_{DNA}. This approach proved to be able to determine residues of the pathogen in extracted DNA samples, without the need of cleanup or purification steps.

(A)

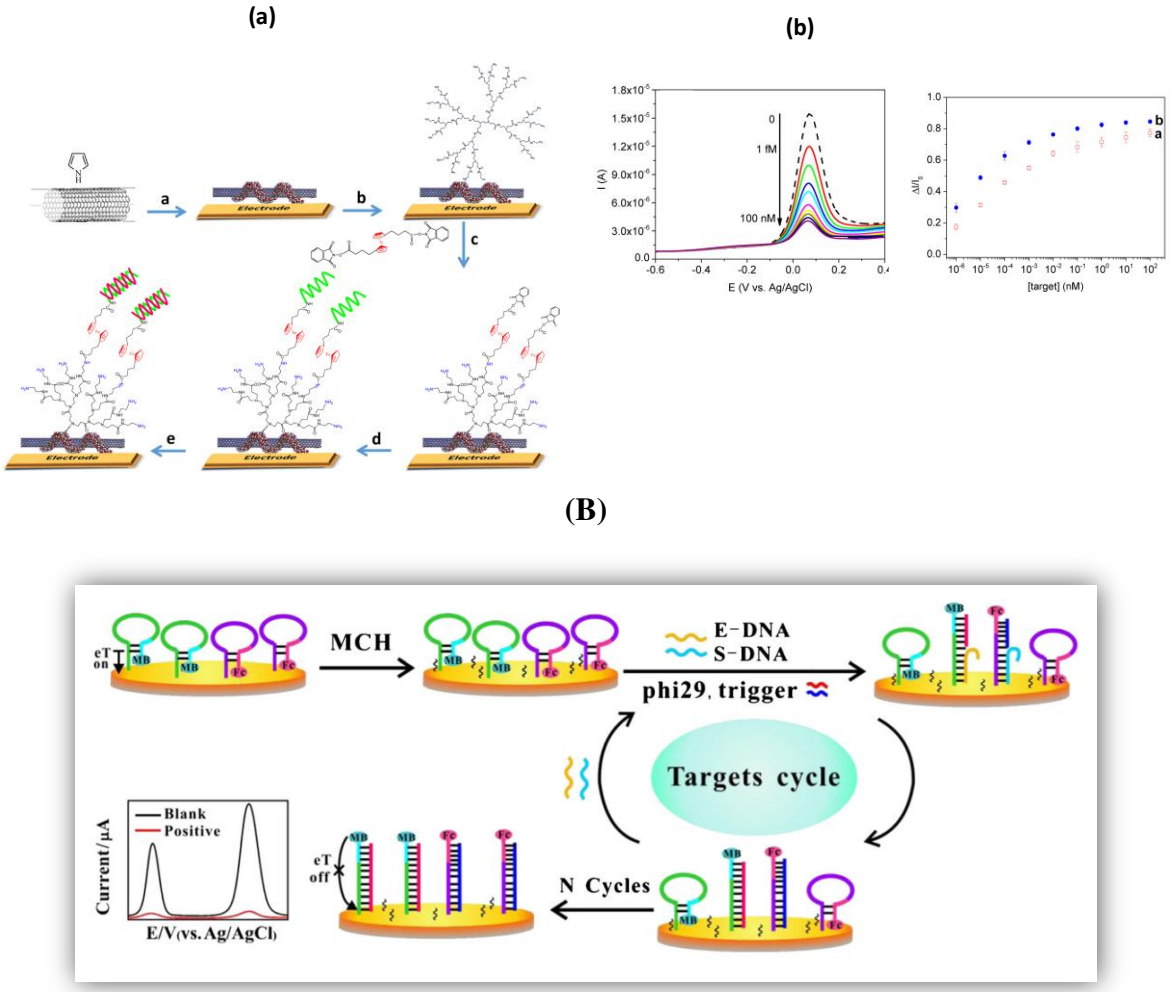


Figure 5: (A) (a) Biosensor preparation using MWCNTs coated with polypyrrole, PAMAM and ferrocene for bacterial DNA detection; (b) SWVs after hybridization of complementary target DNA (1 fM to 100 nM), amplitude=20 mV, frequency= 50 Hz, conditioning time=180 s together with changes of peak current with concentration of (a) DNA_s and (b) DNA_L targets on the MWCNTs-PPy-PAMAM-Fc biosensor (Reprint with permission from Ref.[41], Copyright 2015 American Chemical Society); (B) Multiplexed electrochemical sensor for the sensitive detection of *E. coli O157:H7* and *Salmonella typhimurium* (Reprint with permission from Ref.[42;] Copyright 2017, Springer Nature).

A rapid voltammetric sensor using polymerase-assisted target recycling amplification for the simultaneous determination of *E. coli O157:H7* and *S. typhimurium* by detecting the rfbE gene of *E. coli O157:H7* and gyrB gene of S. *typhimurium* was developed by Guo et al.[42] The sensor

was constructed via self-assembly of the respective hair-pin probes, labeled with methylene blue and ferrocene, onto gold electrode (**Figure 5C**). After hybridization with the target DNA, the primers hybridize with the open-chain hair-pin probes and initiate extension reactions in the presence of polymerase and deoxyribonucleoside triphosphates. This results in the release of the redox labels and the target dissociating from the hair-pin probes. The released target binds to other hair-pin probes to activate new cycles, resulting in enhanced suppression of current, measured at −0.27 V and +0.36 V for detection of *E. coli* DNA and S. *typhimurium* DNA, respectively.

Table 1: DNA based pathogenic sensors.

Analyte	**Method**	**Sensitivity**	**LOD**	**Linear range**	**Ref.**
E. coli	Potentiometry	0.19 nM / mV	1 nM	-	[43]
E. coli O157:H7	EIS	21.485 Ω / log c	0.1 fM	10^{-14}- 10^{-6} M	[44]
E. coli	CA		10^{3} cfu mL^{-1}		[45]
E. coli O157:H7	DPV	2.3501 µA / ln (g mL^{-1})	1 ng mL^{-1}	10^{-9}-10^{-6} g mL^{-1}	[46]
E. coli	DPV	-	6.3 pmol	21- 400 pmol	[47]
E; coli UTI					[18]
E. coli O157:H7 *S. typhimurium*	DPV	-	0.32 fM 0.67 fM	0.5 fM- 5 pM 1 fM - 5pM	[42]
*E. coli*16S rRNA	CA	-	250 cfu mL^{-1}	-	[48]
E. coli			3.6×10^{3} cfu mL^{-1}	-	[49]
S. aureus	DPV	0.469 µA / M	0.244 fM	-	[38]
S. typhimurium	DPV	0.269 µA/lg c	28 cfu mL^{-1}	72-10^{6} cfu mL^{-1}	[50]
Salmonella	SWV	-	0.4 µM	-	[35]
Salmonella	DPV	2.66 µA / log c	10 cfu mL^{-1}	10-10^{5} cfu mL^{-1}	[51]
Aphanomyces invadans	ASV/DPV	-	1 fM	-	[52]
Aeromonas hydrophila	SWV	-	100 f M	-	[33]
Vibrio cholerae	DPV	35.20 nA / ng cm^{-2}	31.5 ng μL^{-1}	100-500 ng μL^{-1}	[53]
Streptococcus Pneumoniae	SWV	0.437 µA / log c	0.093 cfu mL^{-1}	5-100 cfu mL^{-1}	[54]
Pneumococcal protein A	SWV	0.271 µA/ ng mL^{-1}	0.218 ng mL^{-1}	0-8 ng mL^{-1}	[54]
Vibrio parahaemolyticus	DPV	0.425 µA μM^{-1}	2.16 pM	20 pM-20 nM	[25]
Piscirickettsia salmonis		0.86 µA M^{-1} mm^{-2}	0.5 nM	0.5nM-1 mM	[40]
Mycobacterium tuberculosis	CA	-	1 fM	1-100 aM	[39]
hepatitis B virus (HBV)	ASV	-	85 pM	0-500 pM	[55]
avian influenza A (H7N9) virus	CA	-	100 fM	1 pM - 100 nM	[36]

EIS: Electrochemical impedance spectroscopy; ASV: anodic stripping voltammetry; CA: chronoamperometry; SWV: square wave voltammetry; DPV: differential pulse voltammetry.

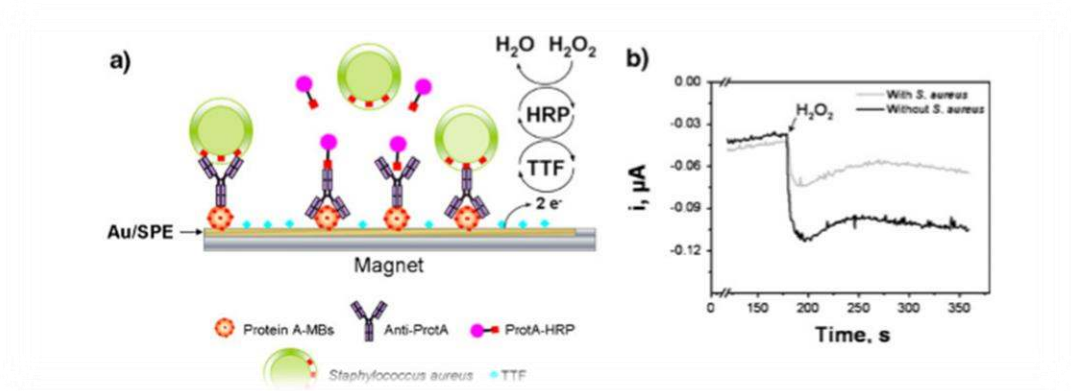
Antibody-based sensors

Antibody-modified sensors allow for fast and sensitive analysis of a large range of pathogens toxins (**Table 2**).[56] Emphasis was on the detection of foodborne microorganisms over the years,[57] and additional examples dealing with fungal pathogens, mycotoxins, viruses and marine toxins are provided. Polyclonal antibodies are widely used, but have often not the mandatory specificity for sensing. Monoclonal antibodies are produced using hybridoma technology using murine hosts., while recombinant antibodies ones are formed using phage display technology. These antibodies are not entirely exploited, while having important advantages such as enhanced selectivity and specificity as well as the possibility to incorporate tags for isolation, immobilization and characterization. When selecting an antibody for pathogen detection, certain characteristics are sought after. It should detect very low cell numbers as this represents a frequent issue in pathogen sensing, and differentiate specific strain.[58]

Gehring et al. demonstrated already more than 10 years ago the interest of biotinylated caprine derived antibodies for *E. coli* O157:H7 detection on streptavidin coated microarray slides and fluorescence-labeled secondary antibodies for captured cell readout.[59] This concept was later applied to electrodes: the coupling of specific antibodies to electrode transducers converting the binding into an electrical signal has proven more attractive than the optical read out, as no secondary antibody to reveal the binding event was necessary.[60] An amperometric sensor for the detection of *S. typhimurim* cells, captured with magnetic bead-conjugated antibodies, after addition of alkaline-phosphatase labeled goat *anti-Salmonella* antibodies using para-aminophenol as substrate was proposed with a sensitivity of 8×10^3 cells mL^{-1}.[60] An electrochemical magneto-immunosensor for the specific detection and quantification of bacteria has also been reported by others.[61] In this case, a competitive immunoassay involving *S. aureus* ProtA antigen labelled with horseradish peroxidase is used (**Figure 6A**). The electrochemical conversion of hydrogen peroxide to water using tetrathiafulvalene (TTF) as electron transfer mediator resulted in a detection limit for *S. aureus* of 3.9×10^{-9} cfu mL^{-1}. Indeed, the literature on the use of enzyme-label amplification strategies to achieve lower detection limits for pathogens dates back to Ruan et al., who reported a detection limit of 6×10^3 cfu mL^{-1} using HRP-labeled secondary antibodies that produce a precipitate of insoluble products on the electrode surface and thus an insulation layer blocking electron transfer.[62]

E. coli O157:H7 cells isolated by commercial immune-magnetic beads modified with antibodies and further labeled with polyaniline nanoparticles modified with monoclonal anti-*E. coli* O157:H7 antibodies by direct physical adsorption allowed magnetically-driven positioning of the samples on screen-printed carbon electrodes.[63] The presence of *E. coli O157* cells inhibits the current flow and used for sensing. This method was used for a variety of target organisms, with the possibility to be a fully transportable system for routine monitoring or bacterial pathogens.[63] Chowdhury has used equally the conducting nature of polyaniline films covalently modified with *E. coli O157:H7* antibodies for the impedimetric sensing of *E. coli*:O157:H7.[64]

(A)



(B)

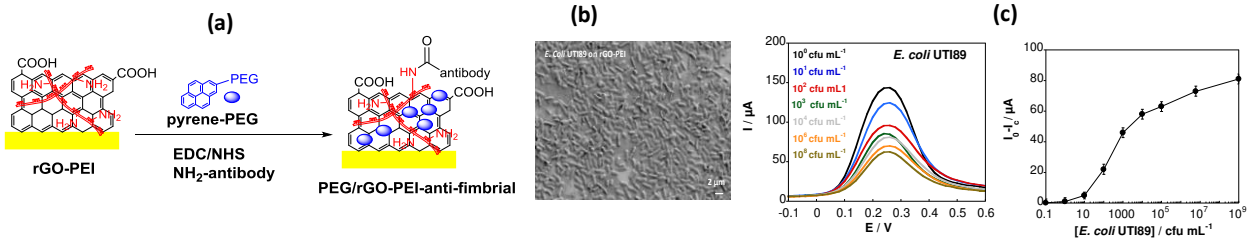


Figure 6: (A) (a) Electro-magneto immunosensor of *S. aureus*, (b) mediated reduction of H_2O_2 with TTF in the presence and absence of *S. aureus* (10^5 cfu mL^{-1}) (Reprint with permission of Ref.[61], Copyright 2012, Springer Nature; (B) (a) Construction steps of immunosensor for *E. coli* UTI89, (b) SEM image of rGO/PEI coated Au electrode after immersion into a solution of *E. coli* UTI89 (10^8 cfu mL^{-1}) for 30 min, (c) Change in DPV signal using $[Fe(CN)_6]^{4-}$ (10 mM)/KCl

(0.1 M), 30 min incubation in *E. coli* UTI89 solutions using the electrode and the corresponding calibration curve (Adapted with permission from Ref. [21]; Copyright 2018, Elsevier).

While such approaches can be easily extended to microarrays, consisting of a panel of pathogen-specific antibodies patterned onto separate positions on microarray slides, and as such represent an excellent candidate for high-throughput analysis of pathogens in miniaturized format,[65] most research in this field is directed on the search for new electrode materials and amplification strategies to enhance the performance of the sensors for bacterial detection. Wang et al. proposed in 2010 a 3D immune-sensor consisting of antibody functionalized 3D foam Ni electrode for the detection of sulfate-reducing bacteria between 2.1×10^{1}-2.1×10^{7} cfu mL^{-1}.[66] Antibody functionalized single walled carbon nanotubes (SWCNT) modified electrodes were successfully applied for the selective detection of *S. aureus* using differential pulse voltammetry. A selectivity assay using *E. coli*, *Bacillus subtilis*, and *S. epidermidis* showed that the sensor was specific to *S. aureus*.[67]

Fei and co-workers constructed recently an electrochemical immune magnetic sensor for *S. pullorum* and *S. gallinarum* using antibody modified Fe_3O_4/SiO_2/AuNPs nanocomposites (AuMNPs).[68] After capture and separation of the pathogens by AuMNPs by an external magnetic field, the formed AuMNPs–*Salmonella* complexes were exposed to HRP-labeled anti-*S. pullorum* and *S. gallinarum* (HRP-Ab2). Modification of SPC electrodes with gold nanoparticles through electrodeposition and using thionine as redox allowed the detection of *S. pullorum* and *S. gallinarum* with a detection limit of 89 cfu·mL^{-1}. In another work by the same group, reduced graphene oxide covered with gold nanoparticles (rGO/AuNPs) was proposed as electrochemical label for *S. pullorum*.[68] some of us have reported recently the interest of electrodes modified with rGO/polyethyleneimine (PEI) thin film via electrophoretic deposition for the sensitive sensing of uropathogenic *E. coli* (**Figure 6B**).[21] While rGO displays high surface area and good electrochemical properties, the presence of $-NH_2$ groups offers a number of opportunities for surface functionalization, such as modificaiton with anti-fimbrial *E. coli* antibodies. To minimize non-specific adsorption, the sensor was further modified with poly(ethyleneglycol) (pyrene-PEG) moieties. The immunosensor displayed a linear concentration range of 1×10^{1} -1×10^{4} cfu

mL^{-1} (R^2=0.995) with a LoD of 10 cfu mL^{-1}. The sensor worked also well in urine, and was able to differentiate *E. coli* UTI89 and UTI89 *Δfim*.

Table 2: Antibody based pathogen sensors.

Pathogen	**Detection Method**	**Sensitivity**	**LOD** cfu mL^{-1}	**Linear dynamic range/** cfu mL^{-1}	**Ref.**
E. coli	EIS		10	$10^0 – 10^3$	69
E. coli	EIS	951.428 Ω /log c	-	$10^3 – 10^8$	70
E. coli O157:H7	EIS	429 W / log c	10^6	$4.36\times10^5 – 3.2\times10^8$	13
E. coli O157:H7	EIS	-	-	$10^2 – 10^7$	64
E. coli O157:H7	LSV	-	200	$10^3 – 10^8$	71
E. coli O157:H7	ECL	867.03 a.u / log c	8	$10^1 – 10^7$	26
E. coli O157:H7	DPV	-	10	$10^1 – 10^6$	72
E. coli O157:H7	EIS	342.99 Ω / log c	1	$10^0 – 10^6$	73
E. coli O157:H7	DPV	0.34 nA/c	3	$3\times10^0 – 3\times10^2$	74
E. coli O157:H7	CV	-	70	$10^1 – 10^5$	75
E. coli O157:H7	EIS	2127 W / log c	100	$3\times10^2 – 3\times10^8$	76
E. coli O157:H7	CV	23.41 µA / log c	15	$3.2\times10^1 – 3.2\times10^6$	77
E. coli O157:H7 *Bacillus cereus*	CV	-	6 40	-	63
E. coli O157:H7 *S. aureus*	EIS	-	100	$10^2 – 10^7$	78
S. aureus	DPV, CV	1.7061 µA / log c	13	$10^1 – 10^7$	67
S. aureus	amperometry	-	1	$10^0 – 10^7$	61
S. aureus	EIS	15000W/log c	10	$10^1 – 10^7$	79
Salmonella gallinarum	CV	0.3418 µA / log c	32	$10^2 – 10^6$	68
Salmonella pullorum	DPV	0.3248 µA / log c	89	$10^2 – 10^6$	68
Salmonella	CA	-	13	$10 – 10^6$	80
Listeria monocytogenes	CA	548.0 nA / log c	-	$10^2 – 10^6$	81
Listeria cells	EIS	7932.6 Ω / log c	160	$1.6\times10^2 – 1.6\times10^5$	82
Streptococcus pyogenes	EIS (Cumulative) EIS (Single-Shot)	- -	- -	10^4-10^7 10^4-10^6	83
Sulfate reducing bacteria	EIS	14.98 W/log c	18	$1.8\times10^1 – 1.8\times10^7$	84
Hepatitis C core antibody	CV	-	12.3 pM	30 pM – 3nM	85
Japanese encephalitis virus.	EIS	-	10 ng/mL	10–500 ng/mL	86

Aptamer based sensors

Aptamers have initiate lately large interest as recognition elements in biosensors replacing other commonly used bioreceptors, mainly antibodies.[87] Aptamers are nucleic acids that bind to

targets with affinities in the micro to picoMolar range, analogous with binding constants of antibodies/antigen interactions. Aptamers offer several advantages making them important emerging ligands for electrochemical pathogen sensing.[87, 88] Their production does not necessitate an immune response in animals, as they are produced chemically by automated nucleic acid synthesis and by an *in vitro* selection process called SELEX (Systematic Evolution of Ligands by Exponential enrichment).[89-90] Aptamers can be chemically modified which permits surface immobilization. Upon target binding, conformation changes occur which helps their detection using electrochemical techniques, where changes in current or potential correlate to analyte-aptamer interactions occurring at the surface. A variety of labels (horseradish peroxidase, glucose oxidase, alkaline phosphatase, ferrocene, nanoparticles, etc) can in addition be linked to aptamers, which in the case of electrochemical aptasensors can result in a single-on or signal-off format.[87] Widely used for sensing of pesticides, [87] one of the first examples for detection of bacteria is the aptamer-based carbon nanotube field effect transistor for *E. coli* DH5a,[91] or the nanoaptasensor by Cella for the detection of anthrax.[92] Binding leads to a change in conductance of the functionalized electrodes. Labib and co-workers established an impedimetric aptamer-mediated sensor to detect living *S. typhimurium* (**Table 3**). The 6-hydroxyhexyl disulfide modified aptamer STYP-3 with a solution K_D=25 mM for *S. typhimurium* was linked over the thiol groups to gold and showed electron transfer blocking using $Fe(CN)_6^{3-/4-}$ as probe. The R_{CT} increases linearly with increasing the concentration of *S. typhimurium* in the range of 10^3-10^5 cfu mL^{-1} with a LOD of 600 cfu mL^{-1} (**Figure 7A**).[93]

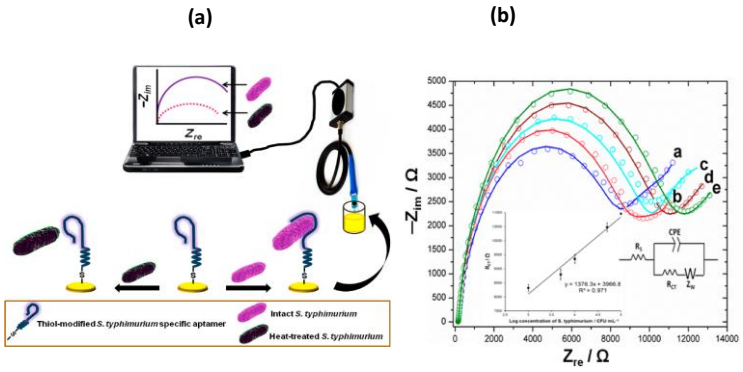
The first potentiometric electrochemical aptasensor for *S. aureus* is that by Zelada-Guillén and coworkers.[94, 22] Single-walled carbon nanotubes were investigated as electrical conducting materials and anti-*S. aureus* SA43 aptamer was used as a recognition element to sense the conformation changes of the aptamer during interaction with *S. aureus* with LOD of 8×10^2 cfu mL^{-1} using covalently linkage strategies and 10^7 cfu mL^{-1} when using a pyrene linking. [22] Two years after, Hernandez et al.[20] proposed a potentiometric aptasensor based on rGO as transducer layers modified with *S. aureus* S1A20 binding aptamer using covalent linkage between the -NH_2 groups of the aptamer and the carboxylic groups of the rGO sheet and non-covalent binding via π-π stacking interactions. Both sensors exhibited detection limits of 1 cfu mL^{-1}, with the covalent linked aptasensor being more easily regenerative. Abbaspour et al. recently designed a dual-aptamer based sandwich using magnetic beads modified with *S. aureus* SA17 aptamer, which

upon interaction with *S. aureus,* were further labeled with Ag NPs functionalized with a secondary *S. aureus* aptamer, SA61.[95] Magnetic separation of the complex, followed by resuspension in HNO_3 for dissolving the Ag NPs allowed the use of differential pulse stripping voltammetry to detect the released Ag^+, which correlates to the concentration of *S. aureus*. A detection limit down to 1 cfu mL^{-1} was achieved with this approach.

More lately, some work was dedicated to the development of an aptasensors for *M. tuberculosis* antigens.[96-97] Bai proposed a coil-like fullerene-doped polyaniline hybrid modified with Au NPs and M. tuberculosis aptamer as a redox and amplification probe for the electrochemical detection of MPT64 antigen in an electrochemical sandwich assay. The proposed sensor displayed high selectivity and was successfully applied for serum analysis of tuberculosis patients.[96]

An interesting impedimetric apatsensor for the detection of *E. coli* O157:H7 was constructed by Brosel-Oliu et al. using three-dimensional interdigitated electrode arrays, consisting of $TaSi_2$ electrodes modified with *E. coli* aptamers (**Figure 7B**).[98] The sensor depicted excellent selectivity to *E. coli* O157:H7 with a detection limit of 290 cfu mL^{-1}. Guo et al. achieved electrochemical detection of *E. coli* pathogens by peroxidase-mimicking DNAzyme (**Figure 7C**).[99] An aptamer-prime probe containing anti-*E. coli* aptamer and a sequence complementary to a circular probe including two G-quadruplex units, was employed as recognition unit, triggering RCA-based polymerase elongation. In the presence of *E. coli* the formation of numerous G-quadruplex oligomers occurs, folding with the help of K^+ and hemin into G-quadruplex/hemin complexes This generates catalytic activity toward H_2O_2.

(A)



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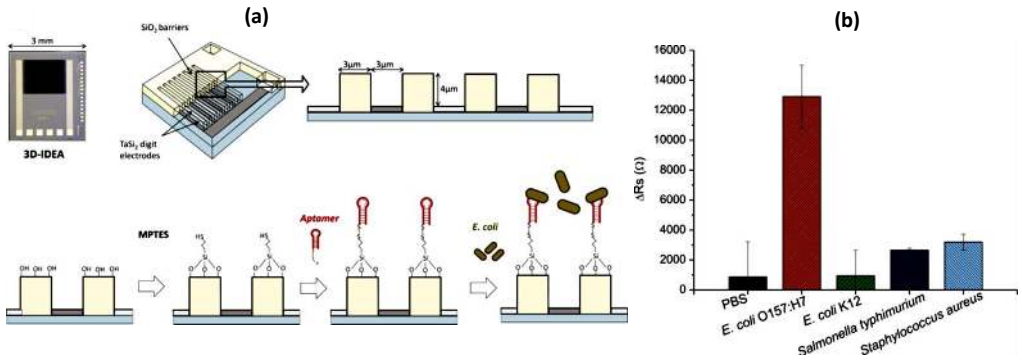

(C)

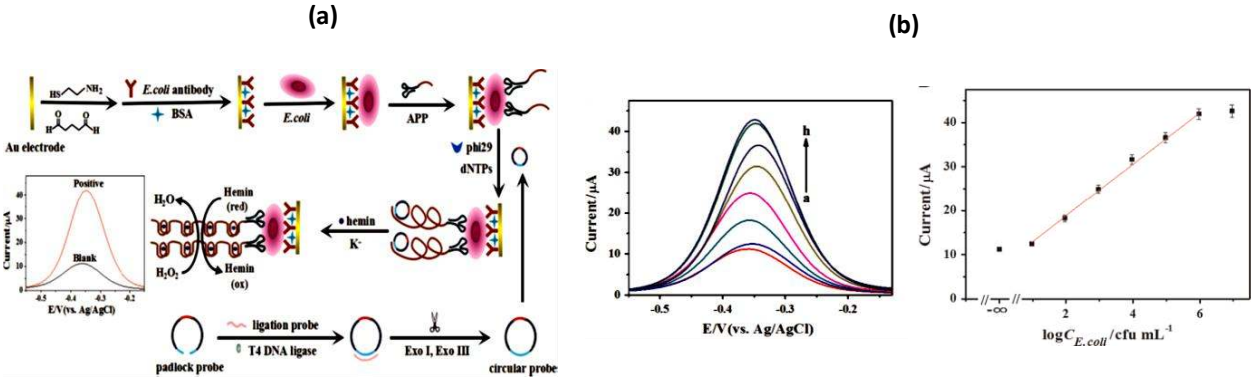

Figure 7: (A) (a) Diagram of an aptamer-based sensor for *S. typhimurium*; (b) Nyquist plot obtained using 1×10^3 (a), 0.4×10^4 (b), 1×10^4 (c), 0.5×10^5 (d), 1×10^5 (e) cfu mL^{-1} of *S. typhimurium:* the insets represent the modified Randers circuit applied to fit data as well as the calibration curve of the sensor (Reprint with permission from Ref.[93]; Copyright 2012, American Chemical Society); (B) (a) Image of 3D-interdigidated electrode and used bio-functionalization steps, (b) Evaluation of selectivity of the aptasensor for the detection of different bacteria strains and control in PBS without bacteria (Reprint with permission from Ref.[98]; Copyright 2018, Elsevier); (C) (a) Illustration of the electrochemical assay of *E. coli* detection based on RCA and DNAzyme amplification; (b) DPV responses to different concentrations of *E. coli* together with calibration curve.- Reprint with permission from Ref.[99]; Copyright 2016, Elsevier);

Table 3. Aptamer-mediated electrochemical pathogen sensors.

Pathogen	Detection Method	Sensitivity	LOD (cfu mL^{-1})	Linear dynamic range (cfu mL^{-1})	Ref.
E. coli	DPV	-	8	9.4×10^{0} –9.4×10^{5}	[99], [9]
E. coli	Conductance	-	-	-	[91]
E. coli O157:H7	EIS	2591 Ω / log c	290	10^{2} –10^{6}	[100]
E. coli O157:H7	EIS	16.1 W/log c	4	5-100	[101]
E. coli O78 :K80 :H11	EIS	-	10	10^{1} –10^{6}	[102]
S. aureus	EIS	-	10	10^{1} –10^{4}	[103]
S. aureus	potentiometry	-	800	2.4×10^{3} –1×104	[22]
S. aureus	potentiometry	-	1	-	[20]
S. aureus	EIS	-	10	10^{1} –10^{6}	[104]
S. aureus	DPV	-	1	10^{1} –10^{6}	[95]
S. aureus	potentiometry	-	8×10^{2}	-	[20]
S. aureus	AS DPV	-	1	-	[95]
S. typhimurium	DPV	100 mA m^{-2} / log c	10	10^{1} –10^{8}	[105]
S. typhimurium	DPV	0.55 mA m^{-2} / log c	10	10^{1} –10^{6}	[106]
S. typhimurium	CV, EIS	841.4 Ω / log c	1	6.5×10^{2} – 6.5×10^{8}	[107]
S. typhimurium	EIS	0.1566 Ω / log c	3	10^{2} –10^{8}	[108]
S. typhimurium	EIS	0.116 ΔΩ / log C	6	10^{1} –10^{8}	[23]
S. typhimurium	EIS	8.6 Ω log c	25	7.5×10^{1} – 7.5×10^{5}	[104]
Clostridium difficile toxin A	CV	0.03 μA / c	1 ng mL^{-1}	1-200 ng mL^{-1}	[109]
M. tuberculosis antigen	DPV	4.4 μA /log c (fg mL^{-1})	0.5 fg mL^{-1}	1-5 fg mL^{-1}	[97]

Antimicrobial peptides (AMP) based sensors

Antimicrobial peptides are synthetic biomolecules targeted against the deactivation of bacteria.[110] Most antimicrobial peptides are positively charged and composed of 10-50 amino acids with a molecular mass of 1-5 kDa. Their positive charge initiates electrostatic interaction with the negatively charged cell membrane of bacteria, but not with zwitterionic membranes of mammalian cells. This selectivity allows their employment as potential recognition elements for electrochemical detection of pathogens. There are several modes of action of AMPs notated as barrel-starve model, carpet model, toroidal model or detergent model.[111] In the barrel-starve

model, the AMP assumes an amphipathic conformation, where the hydrophobic part intermingles with the lipid acryl chains of the membranes. In the carpet model, the peptides are attracted by the anionic phospholipids covering the bacterial surface membrane until saturation with subsequent solubility of the bacterial membrane. The toroidal model is based on the insertion of the peptide parallel to the membrane, causing cell death; the detergent model is grounded on membrane solubilization and cell death. It was from the seminal work of Kulagina in 2005, who highlighted the potential of AMP magainin I (GIGKFLHSAGKFGKAFVGE-IMKS), immobilized on glass, to act as a specific recognition element for *E. coli* O157:H7 and *S. typhimuriym* in a fluorescence based assay, that AMPs got accepted as bioreceptors for sensors (**Table 4**).[112] One of the first electrochemical based sensors using AMPs is that of Manoor et al.[113-114] who demonstrated the ability of magainin I modified interdigitated electrodes for the detection of *E. coli* O157:H7 and *S. typhimuriym* by EIS. Another impedimetric sensor was developed by Li et al. using gold electrodes modified with lipoic acid to which magainin I modified with ferrocene tag was linked covalently using EDC/NHS chemistry.[115] The sensor achieved a LOD of 10^3 cfu mL^{-1} for *E. coli* O157:H7 and a linear correlation up to 10^7 cfu mL^{-1}. At the same time some other impedimetric microsensors for *Listeria monocytogenes* using leucocin A,[116] as well as for *Pseudomonas aeruginosa* and *Streptococcus mutans* using synthetic peptides such as G10KHc AMP and C16G2cys were established.[117] In the case of the *L. monocytogenes* (**Figure 8A**), the antimicrobial peptide was covalently immobilized on microelectrodes via interaction between the carboxylic acid of the peptide and the free amines of a pre-attached thiolated linker.[116] This study underlined the believe that short peptide ligands from bacteriocins offer high selectivity in bacteria sensing with detection limits of 1 cell/µL, a clinically relevant limit. More recently, human lactoferrin modified interdigitated electrode arrays was used to detect *S. sanguinies* in artificial saliva by impedimetric sensing.[118]

(A)

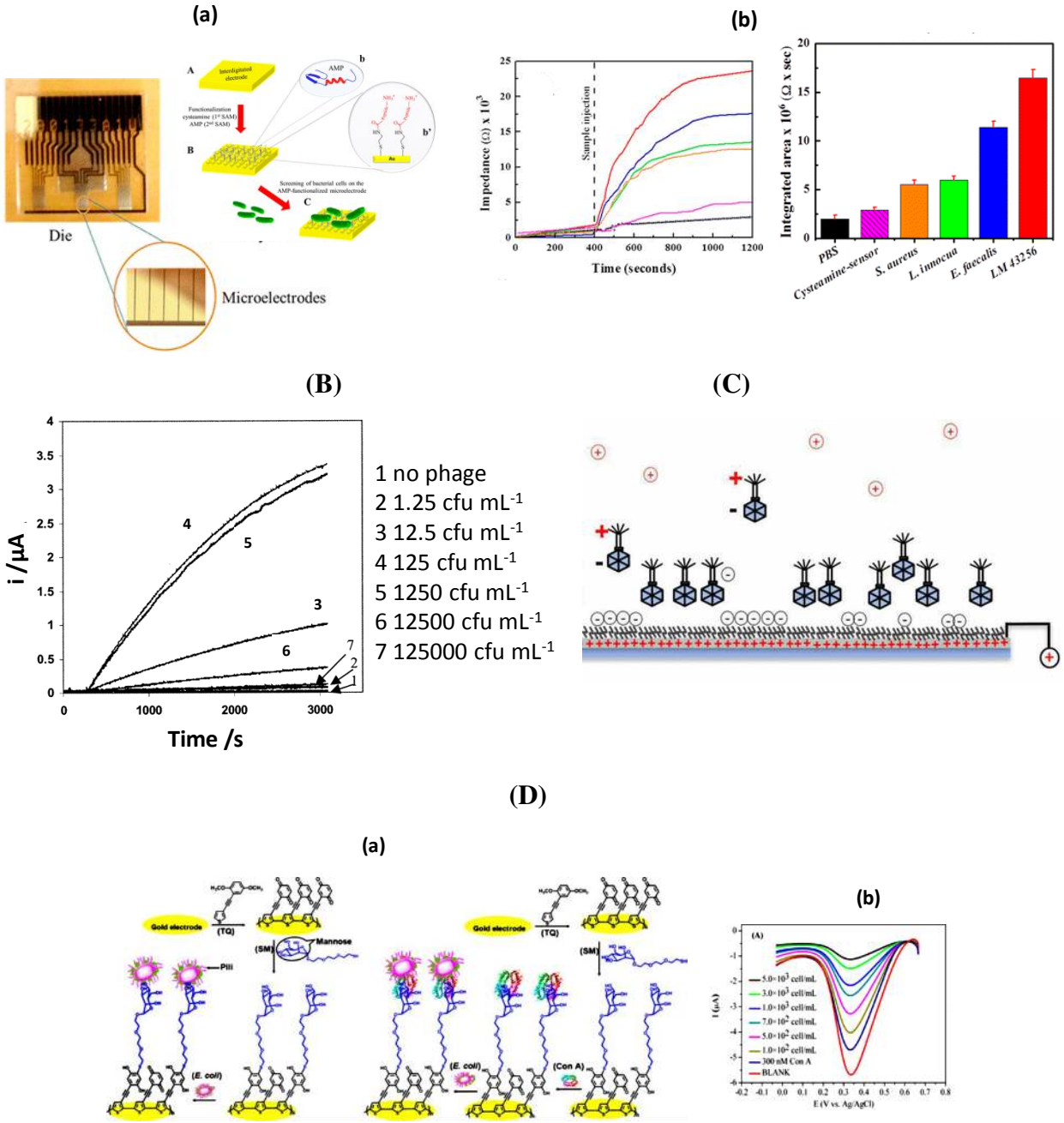

Figure 8. (A) (a) AMP-based biosensor using microelectrode array, (b) Impedimetric response of the AMP sensor to various bacterial species (10^3 cfu mL^{-1}) at 100 Hz (Reprint with permission from Ref.[116;] Copyright 2014, American Chemical Society); (B) Response of β-D-galactosidases activity due to *E. coli* (2.5 ×10^4 cfu mL^{-1}) infected with phage l vir (0-1.25-12.5-125-1250-12500 pfu mL^{-1}) (Reprint with permission from Ref.[119]; Copyright 2003, American Chemical Society); (C) Schematic description of charge-directed bacteriophage immobilization (Reprint with permission from Ref.[120;] Copyright 2017, American Chemical Society); (D) (a)

Different modes of *E. coli* detection based on polythiophene modified electrode with linked glycans (left) for Pili-Mannose Binding and ConA modified interfaces for Con A-mediated *E. coli* binding (right), (b) Square wave voltammetry responses after incubation with Con A and *E. coli* (Reprint with permission from Ref.[121;] Copyright 2015, American Chemical Society).

Table 4. Antimicrobial peptides-mediated electrochemical pathogen sensors.

Pathogen	**Detection Method**	**Sensitivity**	**LOD (cfu mL^{-1})**	**Linear dynamic range (cfu mL^{-1})**	**Ref.**
Antimicrobial peptides (AMP) based sensors					
E. coli O157:H7	EIS		1000	$10^3 – 10^7$	[115]
E. coli O157:H7 *S. typhimurium*	EIS		1000		[113]
S. typhimuriym	EIS		1000		[114]
S. sanguinis	EIS	3.45 Ω / log c	35	$10^1 – 10^5$	[118]
L. monocytogenes	EIS		1000	$10^3 – 10^6$	[116]
P. aeruginosa Streptococcus			100000		[117]
Lectin based sensors					
E. coli	EIS	3650 Ω / log c	75	$10^2 – 10^5$	[122]
			600		[123]
Sulfate reducing bacteria	EIS		1.8	$1.8*10^0 – 1.8*10^7$	[84]
E. coli	EIS		5000	$5*10^3 – 5*10^7$	[124]
Bacteriophages					
E. coli MG1655	CA		100	$10^2 – 10^5$	[119]
E. coli TG1	CA		1	$10^0 – 10^3$	[125]
E. cloi K12	EIS	0.147 Ω/ log c	20	$10^2 – 10^8$	[126]
Salmonella	CA		4	$10^0 – 10^5$	[127]
E. cloi K12	EIS	1.87 Ω / log c	1000	$10^3 – 10^6$	[120]
Carbohydrates					
E. coli ORN 178	EIS		120	$1.2\times10^2 – 2.5\times10^3$	[128]
E. coli	EIS		1700	5×10^4-5×10^7	[121]

6. Bacteriophage based sensors

Bacteriophages (called simply phages) are natural host-specific nanostructured particles. Important advantages of using bacteriophages as ligands is linked to the fast and cheap production. Phages are stable at different pH and temperature. They bind to bacterial surface receptors and insert the genetic material inside the bacteria.[129] The replicated virions are ultimately released, destroying the bacteria and infecting other host cells. Diagnostics based on

bacteriophage is attractive in addition due to the high specificity of phages. towards cell-surface proteins, pili, lipopolysaccharides, lipoproteins.[129] Expression of bioluminescence genes, encoded by bacteriophages (lux-bacteriophage strategy), fluorescence–taged phages which can be combined with immune magnetic separation (label phage strategy), phage amplification strategies as well as or phage mediated bacterial lysis are detection possibilities. One of the first examples, like the work by Rishpon and co-workers,[119, 125] uses phage-specific identification, release of an enzymatic cell marker, following cell lysis for analysis. In their work, they electrochemically determined using screen printed electrodes, biased at 220 mV, the enzymatic activity of α-D-galactosidases, released from *E. coli* K12 and MG1655 cells infected by plaque forming units of virulent λ phage, using p-aminophenyl–β–D-galactopyranoside (PAPG) as substrate (**Figure 8B**). A similar approach, based on the electrochemical detection of alkaline phosphatase, was also reported. Infection of *E. coli* TG-1 with a modified phage, phagemid M13KO7 and pFLAG-ATS-BAP, a gene encoding for alkaline phosphatase, results in the formation of alkaline phosphatase in the space that separates the outer plasma membrane from the cell wall of *E. coli*. The porosity of the cell wall allows p-aminophenol phosphate to enter, facilitating the detection of the activity of the reporter enzyme amperometrically.[125] Next to these assays, surface immobilization of bacteriophages opens up avenues for the development of biosensing platforms. This necessitates however an orientation controlled immobilization, as bacteriophages recognize bacteria via their tail spike proteins.

Different to these works, Shabani et al.[126] studied covalent immobilization of wild-type T4 bacteriophages onto carboxylic acid modified screen-printed electrodes through EDC/NHS amine bond formation. EIS was used to follow the T4 phages-induced lyse of *E. coli* 12 within a period of 40-60 min. After an initial increase in impedance for the first 20 min, attributed to the diffusion of bacteria to the phage-modified electrode, a decrease in impedance was recorded and correlates to bacteria lysis. A linear relation between *E. coli* concentrations and impedance in the range of 10^2-10^8 cfu mL^{-1} was attained, while no significant changes were observed in the presence of *Salmonella*. More recently, Pividori and co-workers[127] used magnetic particles covalently modified with bacteriophage P22 specific to *Salmonella* for phagomagnetic bacteria separation. DNA amplification of the captured bacteria was performed by double-tagged PCR (biotin and digoxigenic signal primer to achieve enzymatic detection via anti-Dig-HRP receptors) through immobilization of biotin-labeled double-tagged amplicon to streptavidin

particles, magnetic capture onto glassy carbon electrodes followed by labeling with anti-Dig-HRP receptors and amperometic detection of formed hydrogen peroxide. The method proved to be able to detect 3 cfu mL^{-1} of *Salmonella* in 4 h.

Some reports concern oriented phage immobilization using charge-directed immobilization[120, 130]. Most phages show an overall negative charge, with a negatively charged head and positively charged tail. This charge variance was used tor controlled immobilization via electrostatic interactions and electrophoretic deposition approaches. [120,130, 131] Zhou et al. described most recently an electrochemical biosensor for *E. coli* B detection using T2 bacteriophages immobilized on polyethyleneamine-modified carbon nanotubes deposited on glassy carbon electrodes (**Figure 8C**).[120] Using electrochemical impedance spectroscopy selective binding towards the B strain of *E. c*oli was achieved with a detection limit of 10^3 cfu mL^{-1}.

Lectin-based sensors

Next to the discussed surface ligands, lectins are known to bind strongly to specific carbohydrate functions on the surface of bacteria and are thus interesting candidates as molecular recognition elements. They are rather easy to produce compared to antibodies and have intrinsic stability. Furthermore, the molecular size of lectins is small resulting in high surface densities of sensing elements with higher sensitivity and low non-specific adsorption.[124] *Conconcanavalin A* (Con A) linking to bacteria with complementary carbohydrate pocket has been mainly used. [122, 123,132] A lectin-based displacement sensor for *E. coli* using ferrocene boronic acid as reporter was reported for example showing a LoD of 600 cells cfu mL^{-1}.[123] Immobilization of Con A on 11-mercaptoundecanoic acid modified gold electrodes was applied for the rapid detection of sulfate-reducing bacteria.[84] More recently, an impedimetric sensor consisting of ConA covalently linked to gold interfaces modified with MUA and dithiothreitol and $Fe(CN)_6^{-3/-4}$ as redox probe, allowed to detect *E. coli* with a detection limit of 75 cfu mL^{-1} over a linear range of 10^2-10^5 cfu mL^{-1} using EIS. [122]

Glycan based sensors

One of the first carbohydrate based electrochemical sensor is that of Heinemann and co-workers.[128] Mannoside and galactoside were immobilized on gold disk electrodes using a SAM *via* a spacer terminated with a thiol functionality to link the ligand to read out *E. coli* ORN 178

impedimetrically.[128] Ma et al.[121] reported on quinone modified polythiophene interfaces which were further glycosylated for bacterial detection (**Figure 8D**) with a LOD to 25 cells/mL over a wide linear range of 10^3-10^7 for pili-mannose and 10^2-10^2 for ConA.[121]

Conclusion and perspectives

The recognition of bacteria and viruses remains challenging and important for ensuring food safety, controlling water and soil pollution, preventing disease outbreaks and ensuring the health state of humans. To date, a plethora of electrochemical sensors for pathogen detection are available. A large amount of these sensors are in line with the main requirements for sensitive, specific and fast analysis. However, only a handful shows high reproducibility and reliable sensing in more complex media than aqueous solutions. Indeed, a major challenge is posed by the potential interfering species such as particulate matter, organic/inorganic contaminants and other biomolecules limiting the life time of the sensors, diminishing the sensitivity of the sensor and ultimately the reliability of the read out. Sensing ligands such as antibodies operate optimally under stringent environmental conditions and may be prone to degradation and thus lowering the selectivity of the sensor. These different challenges have been addressed by taking advantage of robust molecular detection schemes and by replacing natural receptors with aptamers and synthetic templates such as imprinted polymers. The use of bacteriophages as biorecognition elements is an interesting alternative for electrochemical bacteria sensing, but still in its infancy. This is mainly due to a limited range of commercially available bacteriophages, but also it is only lately that dense and well-oriented layers of bacteriophages could be immobilized onto solid transducers such gold.[130]

Currently, the number of strategies proposed for the immobilization of pathogen specific ligands onto electrodes is quite limited. This field might be exploited much further in the future as it is one of the crucial steps in any biosensing based format. While these issues apply to any pathogen sensor, it is evident that the best qualified pathogen sensors should satisfy different requirements such as low cost, portability, miniaturization, easy to use with the possibility of multiplexing.

With the speedy evolution of nanotechnology, an extensive range of nanomaterials are developed for sensing. Integration of these materials and 3D architectures onto electrical interfaces allowed obtaining high sensitive sensors. Among these nanomaterials carbon nanotubes and notably reduced graphene oxide have attracted large interest. Detection using graphene coated sensors

represents an attractive technology and has been proposed by several different research teams in the past The 2D nanostructure of graphene is advantageous for the integration of a high density of ligands in easy manner, often relying on non-covalent π– π stacking and electrostatic interactions. However, this large surface area can also be a limiting factor, fostering interactions with blood serum components and formation of precipitates with red blood cells or serum proteins when sensors are immerged into real samples. A key design feature for real applications is thus to minimize non-specific interactions and improving the non-fouling properties of graphene based electrodes. The possibility of integration of functionalized rGO such as rGO/PEI by electrophoretic deposition, followed by modification with bacterial ligand and integration of anti-fouling units such as pyrene-PEG units using the π– π stacking capacity of rGO resulted for example in highly sensitive and selective electrochemical sensor for uropathogenic *E. coli* UTI89[21] without any amplification. Other nanomaterials such as metallic nanoparticles, notably gold nanoparticles, but also carbon-based nanomaterials have been used not for electrode surface modification but also as electrochemical amplification agents to increase the sensitivity of electrochemical sensors. These sensing schemes are mainly based on sandwich assays, which indeed result mostly in excellent sensing parameters. Their multi-step approach including the synthesis of several nanomaterials together with higher costs due to the sandwich format limit their practical application.

What are the current alternative to electrochemical sensors? The main sensing platforms in competition with electrochemical transducers are currently optical pathogen sensors taking advantage of new optical active nanostructures, new fluorescent nanomaterials (up converted particles, quantum dots, etc) as well as surface resonance technology, optical-fiber and other miniaturizable optical technology to design portable devices. While for a long time they have suffered from detection limits being too high for sensitive pathogen analysis, progress in optical technologies made them increasingly competitive with electrochemical read outs. The often remain costly due to certain optical requirements. Both techniques should be seen as complementary and the adapted method should be reliable and selective to a particular pathogen of interest. Electrical detection using field effect transistors is another appealing alternative. However, these sensors when it comes to pathogen detection and real sample analysis are still in their infancy; more research efforts on anti-fouling strategies by keeping the advantages of an electrical read out need to be addressed to drive these devices to a competing level.

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