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# **Electrochemical techniques for label-free and early detection of growing microbial cells and biofilms**

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## **Abstract.**

Over the past decades, the misuse or abuse of antimicrobial agents to prevent and/or control infections has led to increased resistance of microbes to treatments, and antimicrobial resistance is now a subject of major global concern. In some cases, microbes possess the capacity to attach to biotic or abiotic surfaces, and to produce a protective polymeric matrix, forming biofilms of higher resistance and virulence compared to planktonic forms. To avoid further excessive and inappropriate use of antimicrobials, and to propose new effective treatments, it is very important to detect planktonic microbes and microbial biofilms in their early growth stage and at the point of need. In this review, we provide an overview of currently available electrochemical techniques, in particular impedimetric and voltamperometric methods, highlighting recent advances in the field and illustrating with examples in antibiotic susceptibility testing and microbial biofilm monitoring.

*Keywords* : growing microorganisms, impedance, voltammetry, antimicrobial resistance, antibiotic susceptibility testing, biofilms

## **1. Introduction**

Microorganisms such as bacteria and fungi are ubiquitous organisms playing a key role, that may be positive or negative, in a range of environmental, industrial and biological processes [1-3]. Microbes can exist as free-floating cells (planktonic state). However, they have a strong propensity to attach and multiply on biotic or abiotic surfaces, forming spatially organised dynamic structures, referred to as biofilms. Biofilms, in which cells are embedded into a protective polymer matrix, are more virulent and 10–1000 times more resilient against chemical treatments than free cells [4]. It is estimated that more than 65% of nosocomial infections and up to 80% of chronic microbial infections in the human body are due to bacterial biofilms [5].

The formation of biofilms in industrial facilities, medical devices (e.g., heart valves, catheters, contact lenses) or in directly exposed tissues is therefore detrimental to both industrial activities and human health, with major financial implications [6]. Many biofilms found in the medical field contain several species of bacterial cells, the most common being *E. coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus viridans*, *Proteus mirabilis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* [5, 7-8].

Over the past few decades, the industrial and medical misuse or abuse of antimicrobial agents to prevent and/or fight infections have resulted in an increasing resistance of microbes to treatments. Combined to the fact that only a few novel antimicrobials active against multi-drug resistant organisms are currently in the pipeline [9], antimicrobial resistance phenomenon has been recognized by the World Health Organization as a global health and development threat [10]. Conventional methods for microbial detection, based on cell counting techniques, mass spectrometry, or polymerase chain reaction, have been shown to be sensitive and reliable, but require several hours of pre-culture for enrichment. In addition, they are not adapted to *in situ* biofilm formation monitoring. Microscopic or optical techniques are more suited to investigate metabolic activities of cells in biofilms but require high magnification imaging equipment and/or labeling. To avoid further excessive and inappropriate use of antimicrobials, and to propose new efficient treatments, it is therefore very important to detect microbes/biofilms at their early stage of growth and at the point-of-need. This research area has been intensively investigated and reviewed in the past few years [7, 11-16].

Due to the ionic character of a number of microbial cell components, the capacity of microbes to transfer electrons in course of metabolic reactions, and the electroactivity properties of some compounds produced through metabolic machinery, electrochemical methods are non destructive and particularly well suited for the investigation of microbial systems and their response to external stimuli. Electrochemical techniques combine many advantages, being not only easy-to-use and fast responsive, but also highly sensitive and specific, and easy to miniaturize down to the microscale, providing compact and portable devices ideal for the point-of-need analysis. A number of reviews have been recently published on electrochemical detection of bacteria, covering different aspects, e.g., focusing on particular devices such as sensors and biosensors [17-18], specific techniques such as impedance spectroscopy [19] or scanning electrochemical spectroscopy [20], a specific bacteria such as *Pseudomonas aeruginosa* [21], specific applications of clinical and environmental interest [18, 22-25], or highlighting the interest of coupling optical and electrochemical methods [26]. In this review, we describe recent advances in electrochemical techniques able to probe microbial activity and

response to antimicrobials of either individual free cells or cell aggregates (i.e. biofilm) in the earliest stages of growth. Only label-free techniques will be discussed, and electrodes modified with costly biomolecules (antibodies, aptamers, DNA strands), i.e. biosensors, will not be considered in the review.

After a brief description of the electrical/electrochemical characteristics of microbial cells and biofilms, the pertinence of different electrochemical detection modes (impedimetry, voltamperometry,...) for the rapid detection of planktonic and/or biofilm forms of microorganisms will be highlighted and exemplified. A special attention will be paid to medical applications, i.e., development of advanced AST and investigation of biofilm growth on wounds or medical implants.

## **2. Electrical and electrochemical properties of microbial cells**

Bacteria and fungi are  $\mu\text{m}$ -size objects occurring over a diversity of shapes and geometries, along with highly heterogeneous structures and complex internal composition. They do not behave only as miniaturized chemical and biochemical factories but also as complex electrochemical entities that are highly polarizable when exposed to small electrical field. Although bacteria are prokaryote organisms while fungi are eukaryotes, and despite the fact that their internal contents and cell wall compositions are quite different, they share common electrical/electrochemical properties that can be used for the efficient detection of their ability/incapacity to grow in solution or on surfaces.

Microbes are unicellular organisms that consist of a cytoplasmic compartment surrounded by a cell membrane, further surrounded by a cell wall. As an example, Figure 1a shows the typical structure of a bacterial cell. The cell membrane is composed of a thin lipid bilayer (6-10 nm) with embedded proteins mainly responsible for transport processes of ions, nutrients, and waste across the membrane. This membrane is highly insulating with a conductivity value around  $10^{-7}\text{S/m}$  [27]. The aqueous internal compartment (cytoplasm) contains sub-cellular components such as vacuoles, mitochondria but also many dissolved charged small molecules and biomolecules, that render cytoplasm much more conductive than the cell membrane (up to 1 S/m) [27]. The composition of the outer cell wall, for its part, depends on the type of microbe. Gram-positive bacteria, for example, possess a thick cell wall (50-70 nm) with a highly charged peptidoglycan structure densely functionalized with anionic glycopolymers, resulting in a relatively conductive cell envelope. Due to the reduced thickness of the cell wall (8-10 nm) and the presence of an additional outer lipid membrane layer, the cell envelope of Gram-negative

bacteria is less conductive (Figure 1b). Microbial cells exhibit various shapes, the most common being sphere (coccus type, e.g., *S. aureus*, *Saccharomyces* type yeast) and rod (e.g., *E. coli*, *P. aeruginosa*) [28].

Microbial growth, under planktonic or biofilm form, is highly dependent on a variety of parameters including the availability of nutrients and temperature. Biofilms development onto biotic or abiotic surfaces follows a multi-step, multifactorial and adaptative process commonly represented using the five-phase model shown in Figure 2. In the last phase of the process, the mature biofilm releases planktonic cells able to migrate and spread to new, unoccupied surfaces. [29].

The activity (growth, virulence) of either free-standing or attached microbes can be monitored using a number of electrochemical techniques. Metabolic processes, typically respiration, generate electrons that can be transferred to a conductive surface directly (direct extracellular electron transfer) and measured. However, extracellular electron transfer most often proceeds indirectly via small redox active molecules acting as mediators. These molecules can be added (e.g. resazurin) or are naturally produced by the cells through metabolic processes. Some methods specifically focus on the detection of electroactive biomarkers secreted by biofilm-forming and/or pathogenic bacteria (quorum sensing molecules, virulence factors). The last category of methods measures indirect indicators of cell proliferation or metabolism (e.g., decrease of electrode active area due to cell attachment, change in the external medium conductivity). The electrical properties of microbial cells can be also used to develop advanced concentration techniques, which can be combined with the previous techniques for enhanced sensitivity. The following section provides an overview of these techniques and their potential for the rapid and point-of-care diagnostic of infections.

### **3. Electrical/electrochemical techniques for microbial activity analysis**

*Electrochemical impedance spectroscopy (EIS)*. Among the variety of electrochemical techniques, electrochemical Impedance spectroscopy (EIS) has found widespread applications in cell biology, bioanalysis, and medicine. Due to its capacity to measure slight changes in the electrical properties of electrode interfaces in real-time and without labeling, EIS can provide valuable information on bacterial cell status (alive or dead) and shed light on mechanisms related to a range of cellular processes, in particular microbial growth, metabolism and biofilm formation [19]. In general, EIS applies a sinusoidal perturbation to an electrochemical system across a range of frequencies and measures the electrical response, called complex impedance [30]. A simple electrical description of the electrochemical system can be represented by the

classical Randles equivalent circuit model shown in Figure 3a [31]. The total impedance consists of two components: the electrolyte impedance and the electrode/electrolyte interface impedance, which may be affected by the presence of suspended or attached microbes in several ways. First, microbial growth results in the conversion of uncharged, or weakly charged, substances in the growth medium (e.g., yeast, peptone, and sugar) into highly charged metabolites like amino acids, reducing the solution resistance. Second, the electrolyte/electrode impedance will change due to: (1) the production of electroactive metabolites that facilitate charge transfer at the interface, (2) attachment of biofilm exopolysaccharide matrix on the electrode surface, which impacts the double-layer capacitance and/or charge transfer, (3) direct attachment of microbes to the surface through pili, flagella or membrane proteins, which facilitates charge transfer, (4) attachment of high cell densities to the surface through their outer membrane, and (5) protein adsorption on the surface, that both affect the double layer capacitance and charge transfers at the electrode surface (Figure 3b). Microbial growth usually results in an increase in both conductance and capacitance with time, causing a decrease in impedance, as illustrated in figure 4 [32]. As shown in the figure, impedance changes are detectable only after a minimum concentration of microbes is reached (detection limit).

In this approach (non faradic/capacitive EIS), EIS technique is operated in the absence of a redox probe by applying small perturbations (typically 1-10 mV) to low DC potentials, creating favorable (i.e., non-destructive) conditions for the real-time monitoring of biofilms formation. In presence of a redox probe (faradic EIS), measurement conditions are more disturbing and do not enable continuous monitoring. In this case, cells binding to the electrode surface reduces the electrode area accessible to redox-active molecules in solution, leading to an increase in the charge transfer resistance.

Specific EIS devices dedicated to biofilm detection have been commercialized working at one frequency (Xcelligence©) or two (e.g., BacTrac ©). One frequency, generally under 100 Hz, enables to record the variations of the electrochemical system at the electrode/solution interface, and the second frequency (about 10 kHz) is chosen to measure the variations of conductivity of the culture medium. These instruments are easy-to-use, especially well-suited for rapid in-site industrial applications. Using Xcelligence© device, Ferrer et al. successfully investigated the dynamics of biofilm formation for *S. aureus* and *S. epidermis* clinical strains in the presence of 10 antibiotics exhibiting different mechanisms of action [33]. However, commercial devices are black boxes delivering cell indexes with no capacity to provide a fine understanding of the systems. By measuring EIS across a broad range of frequencies (typically from mHz to MHz), with or without redox probes, and modeling data through appropriate electrical circuits, it is

possible to determine a variety of parameters pertaining to the system which are not accessible another way. Some examples of equivalent circuit models reported in the literature, that take into account electrode configuration and coating as well biofilm growth, are given in the review by Poma et al [25].

Recently, key technological advances in bioimpedance spectroscopy have enabled the development of miniaturized EIS systems under low-cost static or microfluidic formats, that enables rapid and/or high throughput early detection of microbial cells and biofilm growth. Interdigitated microelectrodes (IDMEs) are now one of the most commonly used transducers for microbial growth studies and antibiotic susceptibility testing by EIS. IDMEs consist in a pair of comb-shaped electrodes on an insulating substrate. Compared to conventional macroelectrodes and geometries, IDMEs offer many advantages, such as small required detection volumes, low Ohmic drop, fast response, higher signal-to-noise ratio, and they do not require a reference electrode [19]. Highly sensitive EIS response can be achieved by reducing the gap between the digits. The typical distance is about 1-150  $\mu\text{m}$  and is limited by the microfabrication technique (e.g., screen printing, gravure printing, inkjet printing, 3D-printing). The most common geometries proposed in the literature are square and circular IDMEs. Owing to their small size, IDMEs can be used, as fabricated [34] or modified [35-36], for direct EIS detection of bacterial growth in compact static devices. IDMEs can also be easily integrated and multiply in microfluidic platforms [37], facilitating the incorporation of in-device preconcentration methods such as dielectrophoresis (DEP) for enhanced sensitivity [38-39]. In addition, operating under flowing instead of static conditions offers more realistic conditions for the investigation of biofilm growth dynamics [40].

*DEP technique.* Although sometimes included in the category of electrochemical techniques, DEP is in fact an electrical technique very powerful for focusing and concentrating polarizable objects, such as microbial cells. It has therefore been included in this review. Similarly, impedance technique coupled with DEP, as described in the following paragraphs, is more an electrical impedance technique than an electrochemical impedance technique but has not been excluded, in view of the exciting results recently obtained using these techniques in the application field addressed in this review. The very different electrical properties of the adjacent structures of the cell result in large interfacial polarisations at the boundaries of the structures and confer to the microbial cell a high dipole-induced moment [38]. Applying a spatially non-uniform electrical field to microbes suspended in a medium of different polarizability causes the cells to migrate in specific directions and with specific dynamics, through DEP effect.

Positive DEP (pDEP) occurs when cells move toward the strong electric field regions (i.e., when the polarization of the particles is larger than the polarization of the medium), while a negative DEP (nDEP) effect is observed when cells are repelled toward the weak electric field regions (i.e., when cells polarization is lower). Figure 5 illustrates these phenomena for a non uniform field created by using a DC source and electrodes of different sizes [41]. DC electric fields enable probing cells envelope with high sensitivity but may be damaging to the microbes viability. Therefore, it is generally preferred to generate non-uniformity using an alternating current (AC) source that uses high-frequency electric fields to detect cell internal properties.

To describe the dielectric properties of microbial cells and predict DEP force, the cells are generally considered as homogeneous particles with single or multiple shells. The most commonly used model is the two-shell model, with a low conductive cell wall around a conducting cytoplasm, the dielectric properties of each part being described by conductivity and permittivity parameters (Figure 6 a-b). In medium of moderate conductivity (typically 50 mS/m), increasing the frequency from 10 kHz to 1 GHz enables to polarize the different part of the cells (from the outer membrane to the cytoplasm) and induce nDEP or pDEP behaviours [38]. At low frequencies, the field is blocked by the cell wall and membrane, causing low cell polarization and nDEP behaviour. At higher frequencies, the field gradually permeates through the insulating shell, causing cytoplasm polarization and a shift to pDEP behaviour. Finally, at even higher frequencies, the time for cytoplasm polarization becomes insufficient, while the contribution of permittivity terms starts to dominate, resulting in inflection and even switch back to nDEP behaviour (Figure 6c). The distinctly different sizes, shapes and composition of bacterial cells with respect to each other, cause distinct features within their polarization dispersion spectra compared to the surrounding medium, which enables the DEP force to selectively differentiate the cells.

Coupling impedance methods with DEP enrichment is very attractive for the efficient and selective capture of bacteria at the electrodes and enables to distinguish between healthy and infected/unhealthy cells, with very promising potentialities for routine diagnostic methods, including AST. Several reviews have been recently published on the topics [39,42]. At higher frequencies, the electrical signal applied to measure the impedance flows through the inner part of the cell, reporting information about the inner cell properties, which is better exploited for single-cell cytometry [43]. Figure 7 illustrates two ways of implementing DEP-impedance coupling using IDMEs, in a conventional electrochemical cell (Figure 7a) and in a microfluidic system (Figure 7b). In Figure 7a, the pDEP force is used to capture suspended microbial cells onto the electrodes, The formation of pearl chains of cells linking the electrode gap, results in



increased conductance and capacitance between the electrodes [44]. In Figure 7b two sets of gold IDE arrays are embedded into microchannels. The first set uses p-DEP force to focus and concentrate *E. coli* bacteria (in yellow) into the centre of the microchannel, and direct it to the second sets of electrodes, functionalized with *E. coli* antibodies, for enhanced impedimetric detection [45].

*Other electrochemical techniques.* Among the other electrochemical techniques used for microbial activity investigation and antibiotic-resistance studies, voltammetric and amperometric techniques can provide information on the capacity of microbes or metabolites to transfer electrons to the electrodes [26]. Microorganisms generate electrons through the oxidative conversion of organic matter, but only a few microbial species with the unquestionable ability to transfer electrons directly to an inert electrode have been identified to date [26, 46]. Some microorganisms produce endogenous redox mediators through metabolic or quorum sensing processes (e.g., phenazines, flavins or quinones), that may be used as biomarkers of microbial activity and measured electrochemically. For example, *P. aeruginosa* secrete several QS molecules, among which phenazines (e.g., pyocyanine, PYO, or pyoverdine) act as virulent factors. PYO is an exclusive metabolite produced by *P. aeruginosa* and is therefore a good biomarker for identifying early stage colonization by this pathogen [14,21]. Do et al [47] recently proposed highly sensitive detection of PYO by square wave voltammetry using signal enhancement through nanopore confinement. For microbes unable to produce mediators, as well as to standardize the mediator concentration and the measured current, exogenous addition of electron transfer mediators, such as resazurin, may be used. Amperometric detection of oxygen consumption through microbial respiration has also been proposed for metabolic activity monitoring [23]. Gold and carbon are common materials for electrodes. Engineering electrodes at the micro- and nanoscale enables improving the electroactive surface and electron transfer efficiency for enhanced detection limits. Further, confining bacteria in  $\mu\text{L}$  to nL size volumes can improve sensitivities, which is also ideal for cell growth studies and biomarker determination. New trends in the non destructive and non invasive monitoring of biofilm growth using the promising scanning electrochemical microscopy (SECM) technique can be found in the review by Zhou et al. [20].

#### **4. Recent advances in electrical/electrochemical AST**

A large number of articles have been published on the applications covered in the present review. In the following two sections we will therefore discuss papers published, with only a

few exceptions, in the last five years. The scientific challenges addressed in these recent works relate to the main limitations of current methods, namely cost, portability, ease of use, rapidity and sensitivity.

The use of small electrodes and electrochemical cells ensures portability. Commercial or homemade electrodes produced through conventional photolithographic or printing techniques of lower cost (i.e., screen-printing, or ink-jet printing) on flexible polymer-based or paper-based substrates are often reported. In a very recent work, Domingo-Roca et al. [48] proposed an original device fully printed through the fused deposition modelling (FDM) 3D-printing technology using polylactic acid (PLA)-based materials. PLA is a cheap biosourced and biodegradable material that is easily printable using the FDM technique. The sensor chip consisted in a three-electrode set-up printed from carbon black-PLA conductive composite on a PLA substrate (Table 1). A gel layer containing hexaammineruthenium redox probe in LB growth medium, and then bacterial suspensions, were deposited on top of the electrode surface. The susceptibility of *E. coli* and *P. putida* to several antibiotics was confirmed through faradic EIS measurements at 100 kHz. Following overnight culture, it was possible to determine the bacterial susceptibility in 90 min, much lower than the time required by the standard culture-based AST (24-48 h). This result was improved compared to that obtained previously by the same group (~2.5 h), using a similar gel-modified electrode approach, but multiple commercial screen-printed electrodes (SPE) in a homemade disk device [49] (Table 1). Fluidic devices have been also proposed using EIS detection, combined with enrichment through bacteria capture on antibody modified IDME [50], DEP technique [51], or impedance cytometry [52]. Petruzello et al [53] recently introduced a new microfluidic method able to detect 1-3 bacteria and monitor their metabolism by electrical impedance. The researchers demonstrated that the technique was able to detect susceptibility and resistance within 1h of exposure to antibiotics with three different modes of action. Brosel-Oliu et al [54] reported the detection of *E. coli* growth and its inhibition by ampicillin antibiotics using original three-dimensional IDMEs coated with polyethyleneimine layer for efficient cell capture in the spaces between the barriers of the IDMEs. Adhesion of the bacteria on top of the barriers was avoided by adding poly(N-isopropylmethacrylamide) antifouling agent. Spencer et al [55] recently developed a rapid susceptibility test based on a two sub-microliter chamber device (one chamber for control and one with antibiotics, both equipped with two electrodes at the bottom). The test was successfully evaluated on a panel of 10 bacterial strains and 6 antibiotics using conductivity measurements. The minimum inhibition concentration (MIC) measured at 1h correlated well with a standard

24h microbroth dilution MIC. Despite the number of works using EIS as detection mode, several works [56-59] have been published, focusing on the square wave voltammetric detection of bacterial growth following resazurin addition. Others monitor respiration processes without added mediators [60-61]. (Table 1).

## **5. Recent advances in the detection of biofilm growth on wounds and medical implants**

Colonization of wounds and implantable medical devices by pathogenic bacteria and subsequent biofilm formation can lead to persistent infections, require long-term treatments, and increase morbidity and mortality of patients. To prevent biofilm formation, significant efforts have been made in recent years to incorporate sensors into the medical devices and the wound dressing tissues for *in vivo* detection of bacterial infection at the early stage of colonization. Fontana-Escartín et al [62] recently functionalized isotactic poly-propylene warp-knitted surgical meshes with sensors able to detect bacterial activity without interference of normal eukaryotic cells. The sensors were produced at the mesh surface using a two-step process. The mesh surface was first modified with conducting polymer nanoparticles (NPs) made of poly(hydroxymethyl-3,4-ethylenedioxythiophene) (PHEDOT), and then coated with a homogeneous layer of poly(3,4-ethylenedioxythiophene) (PEDOT) conducting polymer generated by *in situ* electropolymerization of EDOT, using PHEDOT NPs as polymerization nuclei (Figure 8a-i). This approach helped increasing the roughness and electrochemical properties of the surface, resulting in enhanced active surface and electrochemical sensor response (Figures 8a-ii and 8a-iii), with a limit of detection close to 0.4 mM NADH at a scan rate of 100 mV/s. *In vitro* studies demonstrated the capacity of the sensors to detect the dynamics of *E. coli* and *S. aureus* activity by measuring nicotinamide adenine dinucleotide (NADH) coming from respiration by cyclic voltammetry. Zhou et al [63] developed flexible electrochemical sensors for *P. aeruginosa* based on square wave voltammetric detection of phenazines. The sensor consisted in laser induced graphene functionalized with molybdenum polysulfide (MoS<sub>x</sub>) on polyimide substrate (Figure 8b). The sensor performances were assessed in broth, agar, and wound simulating medium. Control experiments performed with *Escherichia coli* bacteria, which are unable to produce phenazines, demonstrated the high specificity of the sensors for *P. aeruginosa*. The sensors enabled real-time monitoring of *P. aeruginosa* biofilms over several days. The results demonstrated the potential of the developed sensors for integration into wound dressings for early diagnosis of *P. aeruginosa* infection. Akhmetzhan et al [64] investigated PYO production through *S. epidermidis* and *P. aeruginosa* growth on drug-eluting coronary stents using differential pulse voltammetry. Comparisons between bare metal

stents and everolimus eluting stents showed that the surface coating and/or the elemental composition of the stent surface affect cell adhesion and biofilm formation in a species-dependent fashion. PYO levels were higher during co-cultures than in *P. aeruginosa* single cultures. In their paper, Foijt et al [65] studied the electrochemical behaviour of Ti-6Al-4V pins in the presence of *E. coli* et *S. aureus* bacterial strains and their potential usefulness as an implantable sensor for detecting inflammation in the human body (typically on the foreign surface of a bone implant). The sensor design and the material used comply with medical device legislation. Changes in impedance values demonstrated both the physical presence of the bacteria, i.e. surface colonisation and biofilm growth, and of their metabolic processes.

## **Conclusion**

As highlighted in this review, the development of fast and low-cost analytical methods able to probe microbes, their activity and response to antimicrobials in the earliest stages of growth and at the point of need is currently the subject of intensive research. In this context, electrochemical techniques are particularly well-suited and competitive compared to other techniques. Emerging approaches in the field have led to propose compact devices integrating microelectrodes and microelectrode arrays of finely tuned geometries into static or microfluidic platforms for confinement and concentration effects, and therefore enhanced sensitivity. In particular, combining EIS technique and DEP enables high enrichment factors and sensitivity for AST application. DEP technique is, however, still at the laboratory-investigation stage. A new microfluidic method has been also recently proposed for the detection of bacteria at the single-cell level using parallel impedance electrical cytometry [66]. Combined to artificial intelligence for easy and automatized data extraction, this method is highly powerful and promising for real-time recognition of antibiotic-susceptible bacteria in mixed samples.

Although robustness of most of the reported methods still needs to be demonstrated in real samples, and should be improved to meet the stringent requirements of health regulations, significant and encouraging progress has been made in the area covered by this review, paving the way for solutions to the antimicrobial resistance crisis.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Captions for figures

Figure 1: The example of bacterial structure (a) and differences in cell structure between Gram-negative and Gram-positive bacteria (b). Adapted from [28]

Figure 2: Conventional representation of a microbial biofilm formation on a solid surface. EPS: exopolysaccharides. Reproduced with permission from [29].

Figure 3: (a) Typical electrical model of electrode/electrolyte interfaces and (b) mechanisms by which microbes may affect the interfacial impedance. : (1) production of electroactive secondary metabolites, (2) attachment of biofilm exopolysaccharide matrix to the electrode surface, (3) direct attachment of the cells to the surface through pili, flagella and outer membrane proteins, (4) attachment of high cell densities through their outer membrane, (5) Protein/macromolecule adsorption on the electrode surface. Adapted from [31].

Figure 4: Typical microbial growth curve (blue) and related impedance response (orange). Adapted from [32].

Figure 5: DEP-induced migration of particles (e.g., microbial cells) in a non-uniform electric field created by electrodes of different sizes. In pDEP, the particle moves to the higher electrical field (left) and in nDEP, the particle moves to the lower electrical field (right).  $F_{Net}$  : DEP force. Reproduced with permission from [41].

Figure 6: Two-shell dielectric models for (a) spherical microbials, (b) Ellipsoidal microbials and (c) Example of polarization dispersion using the two-shell model.  $K_{CM}$ : Clausius-Mossoti factor, which represents the frequency-dependant measure of particle polarizability.  $K_{CM} < 0$  corresponds to a nDEP behaviour,  $K_{CM} > 0$  corresponds to a pDEP behaviour;  $\sigma$ : conductivity,  $\epsilon$ : permittivity (mem for membrane, cyto for cytoplasm). Reproduced with permission from [38].

Figure 7: DEP coupled to impedance using IDMEs and two strategies (a) in a convention electrochemical cell using one set of electrode and (b) in small volume channel using two sets of electrodes to enable sample enrichment and impedance analysis. Reproduced with permission from [38].

Figure 8: Two examples of sensors developed for early detection of bacterial biofilm in the medical field. (a) a surgical mesh is functionalized with PHEDOT NPs/PEDOT (i), the roughness of the surface, provided by PHEDOT NPs, is visualized by SEM (ii) and the CV calibration curve shown in (iii). PHEDOT NPs: poly(hydroxymethyl-3,4-ethylenedioxythiophene) nanoparticles, PEDOT : poly(3,4-ethylenedioxythiophene) ; (b) shows the different steps of fabrication of a flexible sensor aimed to be integrated into wound dressings. LIG: laser-induced graphene . Reproduced with permission from [62] and [63]

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Figure 1

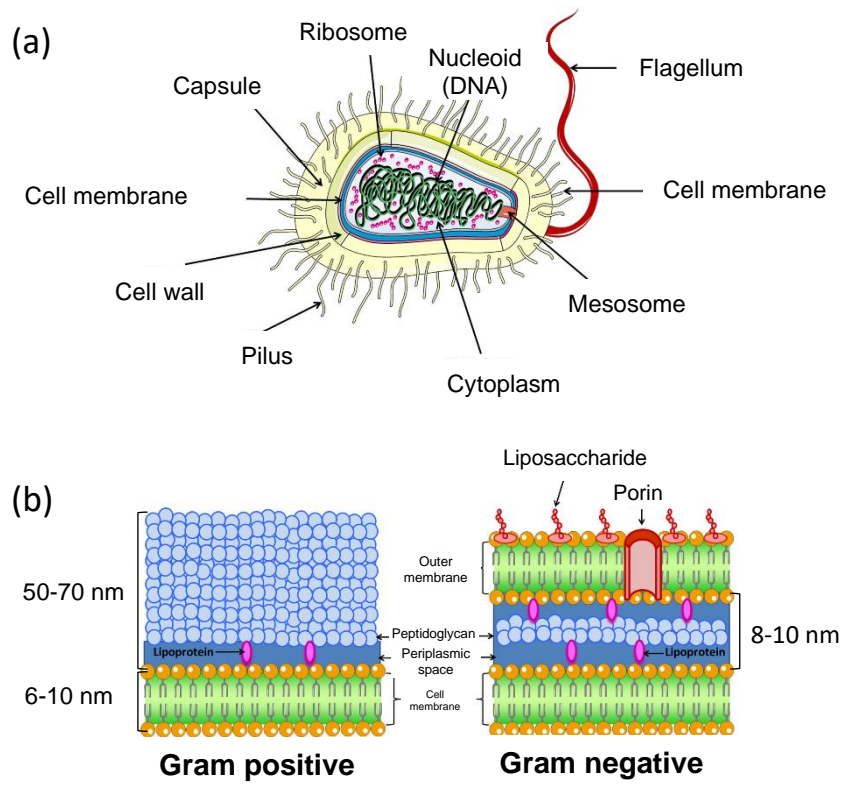


Figure 2

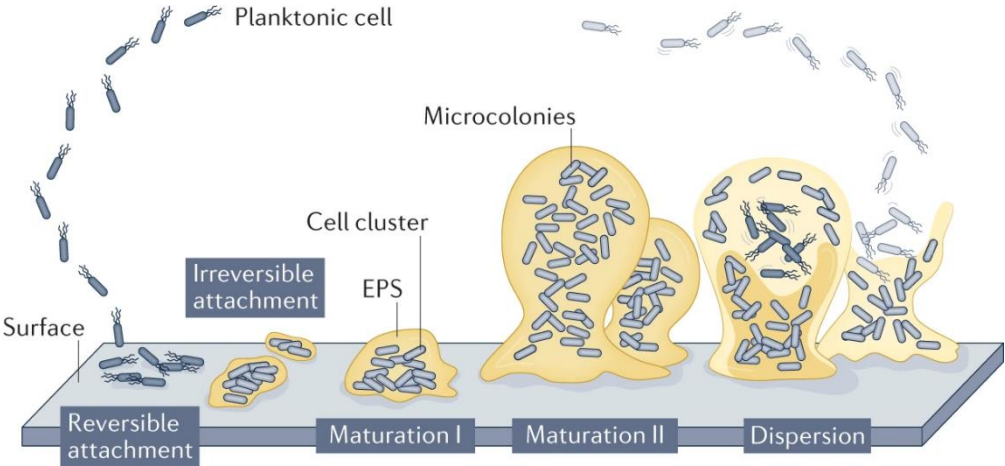


Figure 3

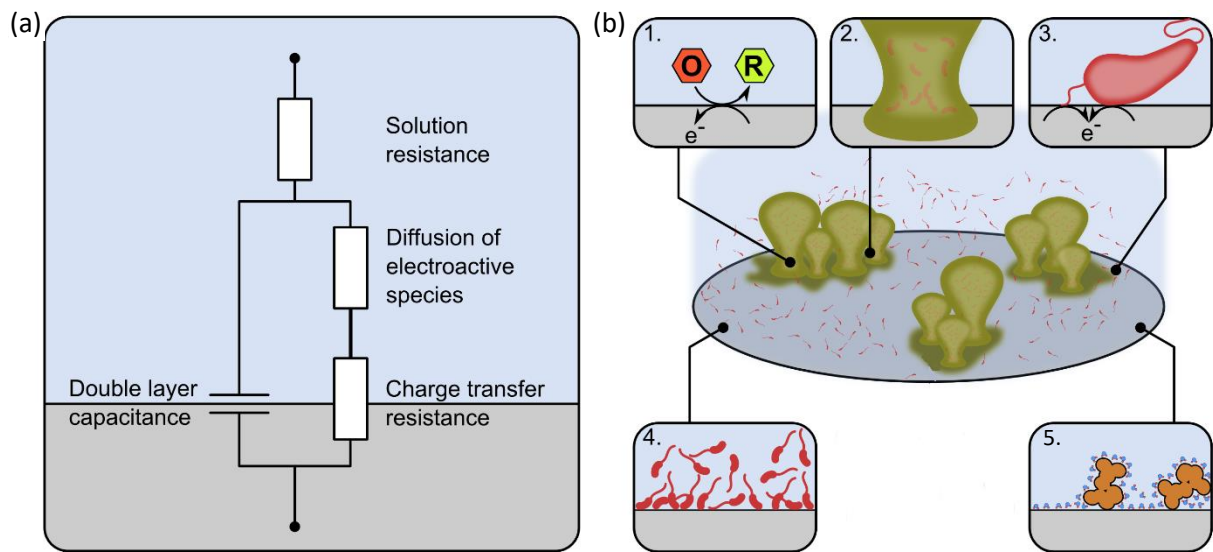


Figure 4

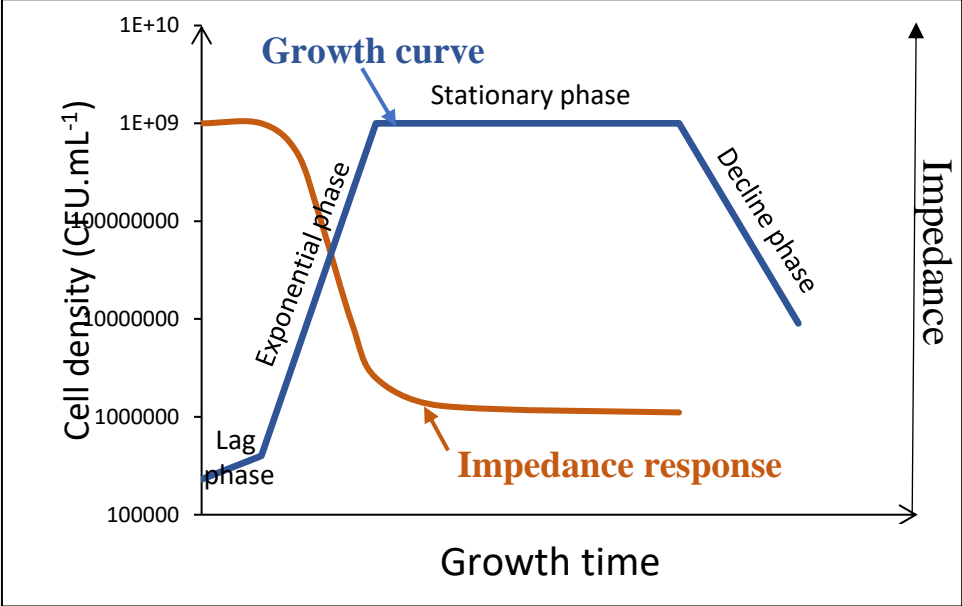


Figure 5

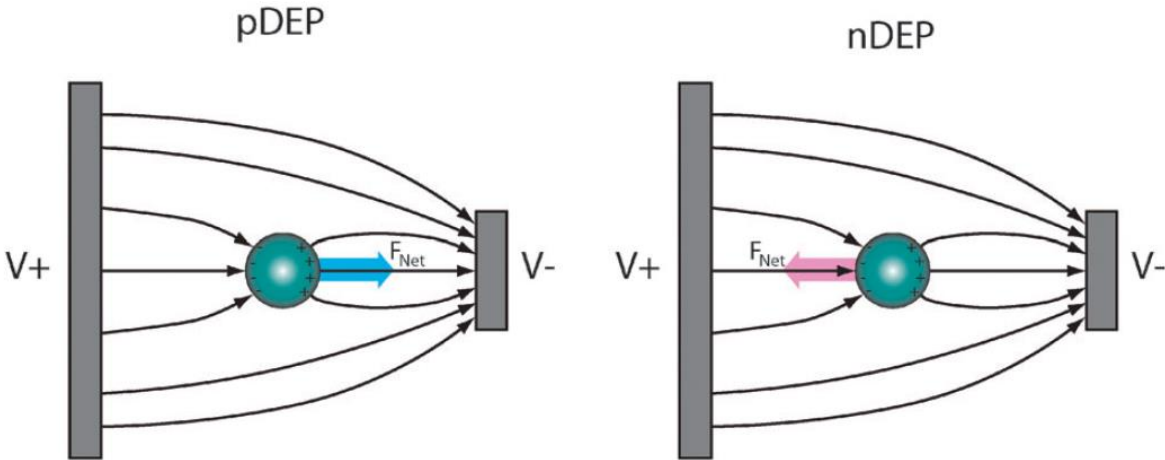




Figure 6

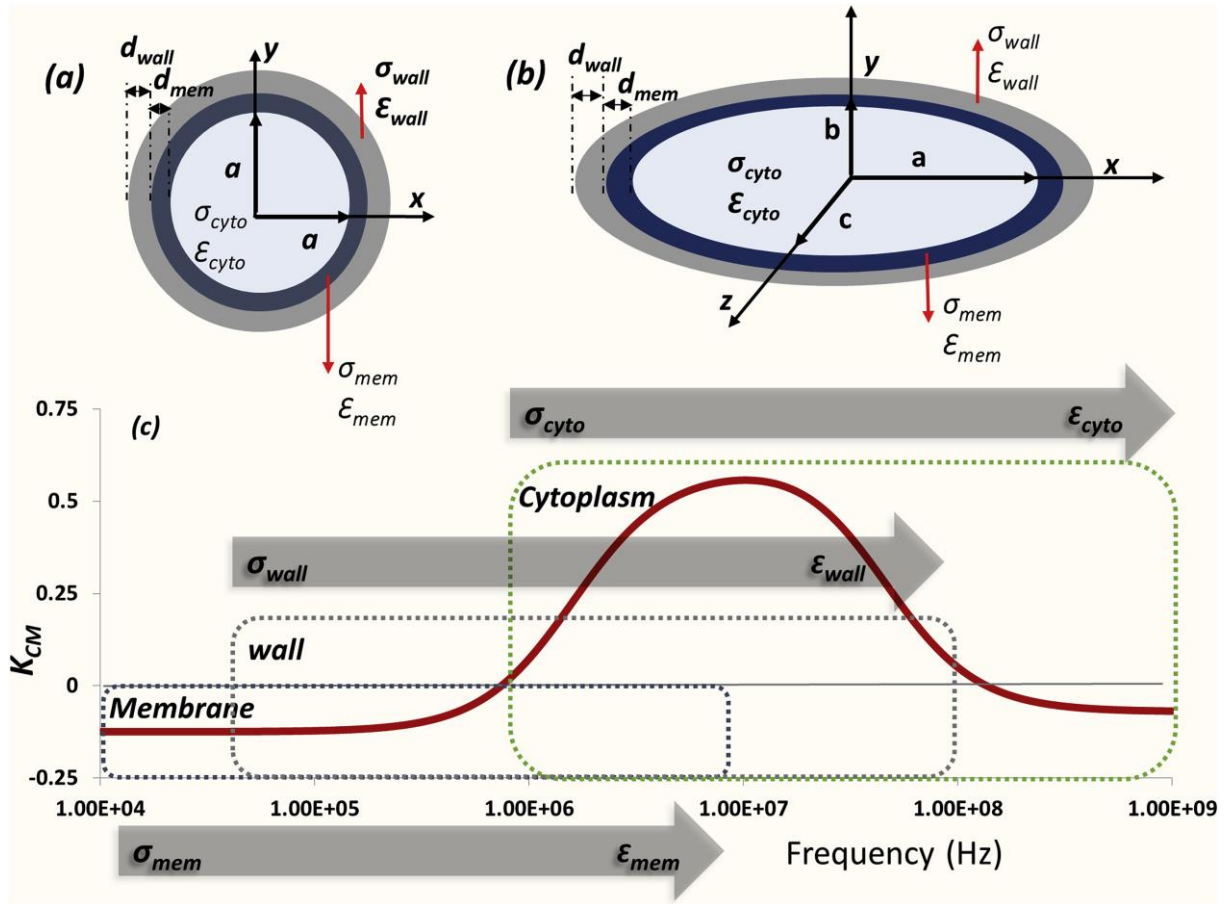


Figure 7

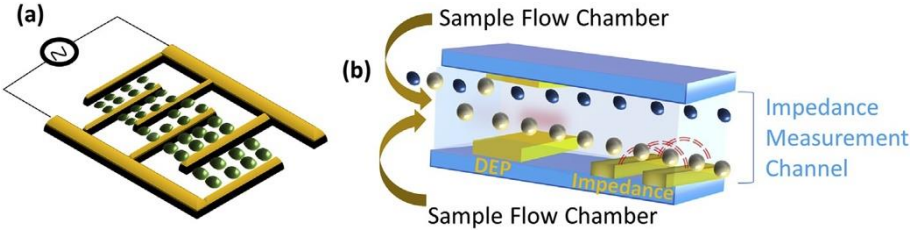


Figure 8

