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Biosensors in Antimicrobial Drug Discovery: Since Biology until Screening Platforms

Juan Bueno*

Bioprospecting development and consulting, Bogotá, Colombia

Abstract

Antimicrobial drug resistance is a current public health problem, which is compounded by the misuse of antibiotics in medical practice and the emergence of Multidrug-Resistant (MDR) microorganisms. Therefore it is necessary to develop new anti-infective drugs and implement new methodologies able to establish the Antimicrobial Susceptibility (AST) in field and the point-of-care. In this sense biosensors is a promising technology that can detect MDR strains and small molecules in various samples, these devices have the advantages that can be miniaturized for obtain portability, rapidity, and cost-effectiveness. The aim of this work is to present the applications of biosensors technology in antimicrobial drug discovery, since cell based biosensors and cell culture on chips, considering metabolic interactions of the microbial world and the pharmacological response to be inhibited by compounds with promising activity with the end of design antimicrobial drug screening platforms robust, automatable and reproducible

Keywords: Biosensor; Antimicrobial susceptibility testing; Antimicrobial drug discovery

Introduction

Antimicrobial resistance is a public health threat, which is being caused by inappropriate use of anti-infective drugs in human and animal health as well as food production, together with inadequate measures to control the spread of infections [1]. Because the use of an antibiotic inevitably selects for resistant microbes, there is a continuing need for new drugs to combat the current generation of resistant pathogens [2]. Frequent misuse of antibiotics leads to bacterial evolution to Multidrug-Resistant Strains (MDR), spreading in human populations. The most commonly identified MDR bacteria are Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant Enterococci (VRE), *Escherichia coli* and *Pseudomonas aeruginosa* resistant to flouroquinolones, *Klebsiella pneumoniae* resistant to ceftazidime, MDR *Acinetobacter baumannii*, and *Mycobacterium tuberculosis* [3].

This public health problem is compounded because there are a few candidate drugs useful for the treatment of infections caused for MDR microorganisms. For that reason in 2010 Infectious Disease Society of America (IDSA) propose that the current antibiotic pipeline problem can be solved by bringing together global leaders to develop creative incentives that will stimulate new antibacterial research and development (R&D), consigned in the 10×20 initiative, that support the developing of 10 new antibacterial drugs for 2020 year [3-6].

In this way, biosensors defined as an analytical device that incorporates biological detecting elements known as bioreceptors integrated with a physical transducer are an important approach to measure microbial cell reporters that can be compatible with High-Throughput Screening (HTS) techniques (Figure 1) [7]. Being classified by bioreceptors in enzymes, microorganisms, antibodies, tissue, organelles and chemoreceptors. Also by transducer types in amperometric, potentiometric, semiconductors, thermometric, photometric and piezoelectric. The combination of these factors (bioreceptor and transducer) composing the fundamental mechanism of development of a biosensor device. The progress in biosensor development is a promising field of application in the antimicrobial research, as useful tool in the discovery of new antimicrobial compounds [8]. Also, biosensors can also be used to develop new diagnostic techniques more specific to detect the emergence of antimicrobial resistance in hospital environment and clinical samples [8-11]. As well as the analysis and detection of microbial food contaminants [12].

The aim of this work is to present the applications of biosensors technology in antimicrobial drug discovery, since cell based biosensors and cell culture on chips, considering metabolic interactions of the microbial world and the pharmacological response to be inhibited by compounds with promising activity with the end of design antimicrobial drug screening platforms robust, automatable and reproducible.

Biosensor Definition and Types

Biosensors are devices for industrial, medical and environmental applications that detect different analytes using biological and biochemical reactions. A biosensor device consists of a biocatalyst (bioreceptor) that can be a cell, tissue, enzyme or an oligonucleotide and a transducer (amperometric, potentiometric, semiconductors, thermometric, photometric and piezoelectric) [13]. Biosensors can be classified by their bioreceptor, their transducer type and the recognition event. Depending of bioreceptor can be classified in [14]:

- Antibody
- Enzyme
- Cell-based
- DNA
- Biomimetic
- Phage

Depending of their transducers in:

***Corresponding author:** Juan Bueno, MD, MSc, Bioprospecting development and consulting, Bogotá, Colombia, Tel: 571-3108323975; E-mail: juangbueno@gmail.com

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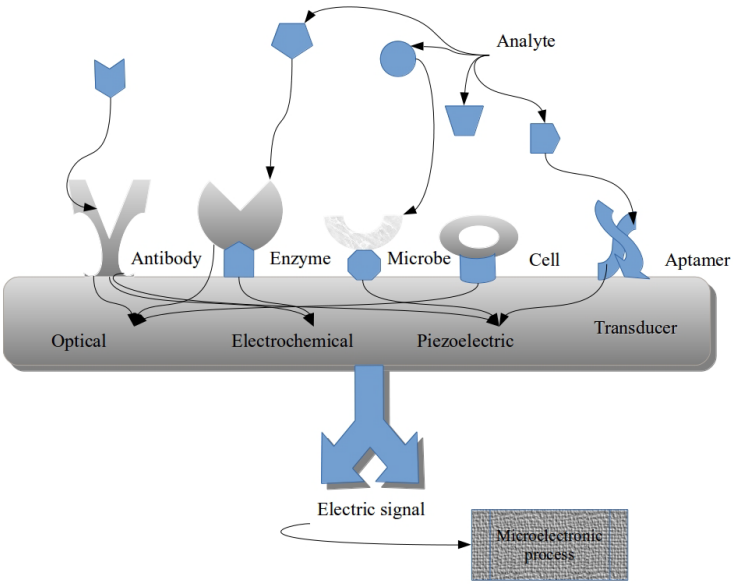

Figure 1: Biosensor principle.

- **Optical**
  - Surface plasmon resonance
  - Raman spectroscopy
  - Fibre optic
- **Mass based**
  - Piezoelectric
    - Quartz crystal microbalance
    - Surface acoustic wave
  - Magnetoelastic
- **Electrochemical**
  - Amperometric
  - Potentiometric
  - Impedimetric
  - Conductiometric

In this review will classify the biosensors described in two categories as are Antimicrobial Susceptibility Testing (AST) and antimicrobial drug bioprospecting, looking for devices and methods necessary for antimicrobial drug discovery.

Antimicrobial Susceptibility Testing

Currently, the methods for Antimicrobial Susceptibility Testing (AST) have some limitations as are the requirement of viable organisms from clinical sample and their processing prior to testing; as well as the few amount of microorganisms standardized, the reproducibility of the results obtained, time to results, and cost. Although new methodologies have been developed, the disk diffusion method and broth microdilution test continue being the standard reference methods with than others AST methodologies should be validated both in vitro assays and clinical studies. An ideal AST have to predict quickly and reproducible success of anti-infective therapy and determine the antimicrobial activity of new drugs by obtaining of MIC (Minimum Inhibitory Concentration) value [15]. For this reason the use of biosensors in AST development is a highly sensitive tool for detection of microbial growth that can reduce the time of a diagnostic procedure and can be used in field (Table 1) [16].

Magnetoelastic elastic biosensor

In magnetic sensors for bioassays, the use of beads and nanoparticles with surfaces functionalized for biomedical interactions make them an indispensable tool for develop quantitative experiments (Figure 2) [17]. In this way Asynchronous Magnetic Bead Rotation (AMBR) biosensors have been used in AST platforms for their ability of detect the growth of individual bacterial cells at an approximate concentration of 50 cells per drop with 80-nm sensitivity to the cell length and determinate MIC of streptomycin and gentamicin against *E. coli*. The basis of this system is a viscometer that measure bacterial growth on self-assembling magnetic microbeads that rotate in a magnetic field in which the rotational period of magnetic sensor is indirectly proportional to the resistance of the object in the fluid (drag coefficient), as are broth medium with bacteria and alone, as well as antimicrobial drugs in several dilutions, but increases with the cell volume, can also be adapted in a microfluidic platform (Figure 2) [18-21]. Also with respect to other sensors, magnetoelastic elastic sensor has the advantages of to be inexpensive, simple and easy to manufacture. Equally, the magnetic field that uses this device can be evaluated remotely and wirelessly, which is useful for screening both liquid culture media as air environments [22].

Electrochemical biosensor

Electrochemical sensors measure the changes of electrical parameters in relation to modifications of chemical properties. Basically, a chemical reaction produces an electrical signal at the electrode by a modification in current, potential or conductivity that is detected by transducer [23]. These biosensors can be used for detect enzymes, nucleic acids, antibodies, whole cells, and receptors, being the enzymes the most common analyte [24]. The major advantages of these techniques include simplicity, low cost and possibility of on-site

Biosensors in antimicrobial susceptibility testing												Biosensors in antimicrobial drug prospecting
Magnetoelastic biosensor	**Electrochemical biosensor**	**Optical biosensor**	**Acoustical biosensor**	**Immunosensor**	**PCR-electrospray ionization mass spectrometry**	**Bacteriophage biosensor**	**Whole-cell biosensor**	**Biofilm biosensor**	**Fluorescent biosensor**	**Nanosensor**	**Microfluidic**	
Asynchronous magnetic bead rotation (AMBR) biosensors	Graphene FET device	Surface plasmon resonance (SPR) biosensor	Bulk acoustic wave (BAW) sensor	Cell phone–based microphotometric system	Ibis T5000™ Biosensor System	FASTPlaque-TB™	Bacteria expressing the luciferase operon	Electro-active biofilm (EAB)	Green fluorescent protein (GFP)	Superparamagnetic iron oxide nanoparticles	AC electrokinetic technique	RNA-aptasensor
	Antimicrobial peptide magainin I	Fiber-optic biosensors	Surface acoustic wave (SAW) sensor	Gold nanoparticle (AuNP) colorimetric probes	PLEX-ID BAC™ detection assay	PhageTek MB™	Vibrating cantilevers with bacteria fixed	Microbial fuel cell (MFC) biofilm biosensor	Cecropin P1 fluorescently labeled with Cy5	Dextran-coated gold nanoparticles	Microfluidic agarose channel (MAC) system	*Bacillus subtilis* with luciferase reporter gene
	16S rRNA probes							AHL biosensor strains			Optofluidic biosensors	
	AptaVISens-B											
	AptaVISens-V											
	Dielectrophoresis (DEP)-AST											

Table 1: Biosensors used in antimicrobial drug discovery.

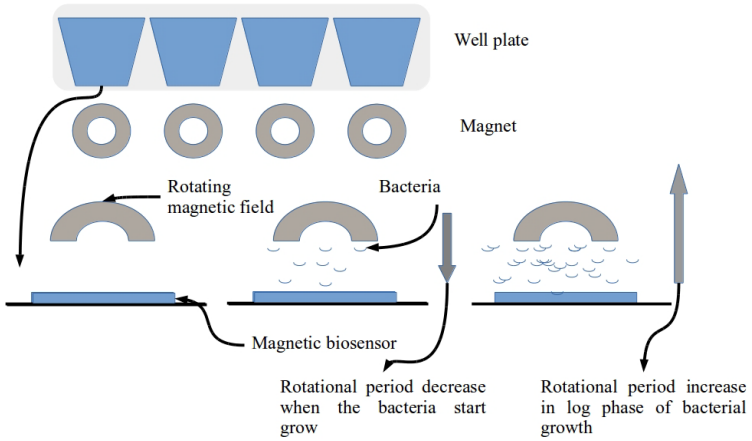

Figure 2: Asynchronous Magnetic Bead Rotation (AMBR) biosensors.

analysis [25]. Also a great advantage is the possibility of interaction between the electrodes and the material to be tested without causing damage to biological systems involved [26].

Using this technology, various AST methods have been developed, between them graphene FET device that is a novel graphene sensor array, which use a material with unique electric properties which allows it to handle much higher frequencies than the silicon, this ultrasensitive recognition element detect the modification of device in front of the changing composition of nutritional components of a culture medium and permits measure the bacterial growth of *E. coli*. Graphene FET device shows to be a promising approach for the development of diagnostic and evaluation of new drugs methods faster and effective drugs [27].

Equally, is necessary the development of robust portable biosensors for the detection of pathogenic bacteria in field, these devices could impact several areas since water quality monitoring until drug testing [28]. In this order of ideas, the use of antimicrobial peptides that exhibit activity against pathogenic bacteria can be a model of detection for new diagnostics methods and AST protocols, also synthetic biology discipline allows design specific proteins for these biosensors [29]. In the electronic detection of infectious agents have been developed a electrode based on antimicrobial peptide magainin I, in this device magainin was immobilized on gold microelectrodes and exposed to various concentrations of *E. coli* showing a detection limit of one bacterium per µL that has clinical utility and adapted in a microfluidic flow cell can be an on-chip with monitoring in real time of bacterial growth, this opens a door for future medical and environmental applications [30].

Other interesting approach have been developed by Mach et al., which combinates 16S rRNA probes, that uses as biomarker of bacterial growth precursor rRNA (pre-rRNA), due to the ratio of maturation of pre-rRNA to rRNA is low during stationary phase and high during log phase, this process is possible to adapt it in an electrochemical

sensor and the rRNA probes can be captured on electrode surfaces in a biosensor-based AST (b-AST) as device for diagnostic and treatment of urinary tract infections. In this case 16S rRNA works as a bacterial growth marker [10,31]. In field this biosensor was tested in pathogen detection on 109 urine samples with specificity and positive predictive value of 100%, likewise pathogen detection sensitivity was 89%, with a 76% of negative predictive value [32]. Subsequently this research group developed a biosensor with integrated pathogen 16S rRNA as oligonucleotides detector and host lactoferrin in antibody sensor, which achieved for pathogen detection a specificity of 97% and a sensitivity of 89% on 113 clinical urine samples [33]. Also, a Self-Assembled Monolayer based electrochemical sensor (SAM) has been proven as a new platform for diagnostics of infectious diseases in point of care. This electrochemical sensor have the ability of increase of sensitivity of pathogen 16S rRNA hybridization assay and reduce incubation time using electrokinetic enhancement with a constant flow from anode to cathode [34], equally was able to establish the MIC value of rifampin, ciprofloxacin and chloramphenicol and could be a promising AST method [35].

Likewise is possible use specific DNA aptamers in an impedimetric sensor for bacteria as AptaVISens-B. This impedimetric sensor is integrated to gold nanoparticle (GNP-SPCE) and can detect *Salmonella typhimurium* in a limit of 18 live cells in 30 μL, which can useful for infectious disease diagnostic. This technology can also be used for viruses (AptaVISens-V) with the ability of detect 60 virions viable of vaccinia virus in one microliter [36,37].

On the other hand, the use of Dielectrophoresis (DEP), which measure dielectric properties of bacteria and their changes under antibiotic treatment, have the ability of establish the number of viable microorganisms by determining of the crossover frequency (cof) (point in where in electric field DEP force totally turned into positive DEP force). A DEP- AST method with *E. coli* and the antimicrobial drug cephalexin was carried out, showing that cof value reduced while increasing the concentration of antibiotic. This platform was able to distinguish between treated and untreated cells at time of 60 minutes, being a methodology that can be promising for the development of new AST devices [38].

But is very important take in consideration that is possible determines MIC values employing impedance (electrical resistance) AST methods, that measures the effects of bacterial metabolic process on the conductance/impedance of the microbial suspension at a certain frequency, this method have the potential of to provide results in 4 hrs. about MIC of bacterial strains and the mode of action (static/cidal) of the antimicrobial agents [39]. This effect of bacterial growth in electrical signal as well as the effect of electromagnetism in microorganisms is factors to be considered in the design and development of these platforms [40]. Although it is necessary to consider the following disadvantages of these techniques as are low sensitivity under 10^6/mL cell concentration and presence of non-specific binding [41].

Optical biosensor

The use of optical biosensors has been described to over 3,000 scientific communications in pharmaceutical and diagnostic research. Covering all kind of biochemical reactions [42]. An amount of analytes can be detected using optical signal transduction as are cells, cell receptors, carbohydrates, antimicrobial peptides, and siderophores [43]. Between them Surface Plasmon Resonance (SPR) biosensor is an optical system where plasmons are excited and transmitted across a coating with ligand that interacts with an analyte in a fluid [44]. This method can be useful for study interactions compound-microbial membrane cell that can establish antimicrobial action of bioactive molecules [45,46]. Also can be adapted with antibodies for develop immunosensors for detection of virus, bacterial and fungal cells [47]. Chiang et al., have reported the development of an innovative AST method utilizing SPR, in where susceptible and resistant strains of *E. coli* were evaluated against ampicillin, the results have shown that SPR biosensor can perform a more faster AST method for obtain quantitative data of antimicrobial drug resistance [48]. The greatest advantage with the use of this type of sensor is their ability of nanomolar detection [49]. But has the disadvantage of non-specific binding on the surface when biological molecules are collected on the sensor, particularly in metallic surfaces [50].

Other kind of optical biosensors are fiber-optic biosensors that employs an optical fiber for develop the biological detection and offer advantages as are: not be affected by electromagnetic interference, small size that allows taking measurements under various conditions, stable calibration and ability to simultaneously analyze multiple analytes [51,52]. However, they have presented drawbacks as are: only works with appropriate reagents developed for this purpose, the light may interfere with the results obtained and present slow response time [51]. In this way, a tapered fiber optic sensing device for in situ real-time monitoring of bacterial growth of *E. coli* have been developed, with a detection limit of 10 bacterial cells and can measure microbial growth in 1.6 hours, making it an alternative to other methods such as colony counting and optical density [41].

Acoustical biosensor

Acoustic wave biosensors use mechanical acoustic waves for signal transduction. Currently, are classified in Bulk Acoustic Wave (BAW) sensor, Surface Acoustic Wave (SAW) sensor and Acoustic Plate Mode (APM) sensor [53]. For development of new AST methods have been used a BAW sensor for bacterial growth with the end of obtain MIC values as well as time kill curves for establish static/cidal activity of antimicrobial agents against *E. coli*, *S. aureus*, *Proteus vulgaris*, *Pr. morganii* and *Pr. mirabilis*, presenting greater accuracy than broth micro-dilution method [54]. For the study of interactions between biomolecules and microbial cell membrane, that can predict antimicrobial activity and action mechanisms, SAW biosensors show the ability of measure mass and viscoelastic properties of microbial monolayer on sensor surface, by determining of impedance changes determined by microbial metabolism [55], in that way interactions on bacterial membranes of gallidermin and vancomycin was studied with SAW biosensors for identify the binding of antimicrobial peptides in bacterial cells [56,57]. Also, SAW biosensors have achieved to measure the growth in 7 hours of *E. coli*, providing the ability to perform monitoring of microbial growth in real time and can be adapted to a remote query wireless for use in dangerous environments [58]. The major disadvantage of this device is the joint use of both monoclonal and polyclonal antibodies as bioreceptor due to its high cost, low availability and laboriousness in the immobilization on the sensor [22].

Immunosensors

Immunosensors are based in interaction between antibodies and antigens using polyclonal, monoclonal and recombinant antibodies for recognition of foreign molecules, being used in immunoassays development with high specificity and sensitivity [59,60]. These biosensors can be used for monitoring microbial growth, using a surface coated with anti-*Aspergillus niger* polyclonal antibodies was possible quantify immobilized fungal spores of *Aspergillus niger* in a

biosensor on silicon micro fabricated cantilever arrays in real time, which permits measure spores in environments [61]. On the other hand, microchip enzyme-linked immunosorbent assays with specific anti-bacteria antibodies and antibiotics concentrations, can be adapted to cell phones with camera integrated for perform rapid AST in the field. Showing a low cost and portable diagnostic device. This cell phone–based micro photometric system is of great applicability in high-burden infection areas to control infections caused by MDR microorganisms [62]. Equally gold nanoparticle (AuNP) colorimetric probes are other another adaptation to develop low-cost immunosensors on paper substrates, which are thermostable and useful in pathogen detection [63]. Also, this strategy can be employed in bacteria mass quantification, attaching the enzyme β-galactosidase to gold nanoparticles, with which can be detected 1×10^2 bacteria/mL in solution and at 1×10^4 bacteria/mL in a strip format [64]. The major drawback of this method is that requires specific antibodies for each microbial species tested, this includes the use of a large number of reagents of low stability under extreme conditions, increasing costs [65].

PCR-electrospray ionization mass spectrometry

Microbial identification and genotyping are necessary in public health for infections diagnosis and surveillance of antimicrobial drug resistance. In this sense multiplex biosensing provides screening platforms with high-performance, as the coupling of nucleic acid amplification to electrospray ionization mass spectrometry and base-composition analysis in a PCR–Electrospray Ionization Mass Spectrometry (PCR-ESI/MS), which has the ability to obtain a rapid diagnosis of clinical samples [66], the technique is marketed under the name of Ibis T5000™ Biosensor System, this technology is capable of performing 1500 PCRs in 24 h and identify around all known human pathogens as well as detect genes involved in antimicrobial drug resistance [67,68]. PCR-ESI/MS require initial extraction and amplification of nucleic acids for analysis, subsequently mass spectrometry determines the mass and base composition of samples, being more faster and robust that traditional cloning and sequencing, the major disadvantage of this method is sample preparation because for each organism should establish a proper protocol analysis and extraction of nucleic acids [69].

Equally, using the same principle was developed PLEX-ID BAC™ detection assay that employs 18 primer pairs into multiwell plate for detection of bacteria and *Candida species*. Also, can detect genes associated with resistance to vancomycin, carbapenems and β-lactams. Their biggest advantage is the ability to diagnose polymicrobial infection [70,71].

Currently, (PCR-ESI/MS) has become more robust platform with the ability of detect bacteria, fungi, viruses, and protozoa making it a promising tool in the clinical laboratory and to the attention of outbreaks and public health threats [66].

Bacteriophage biosensor

Phage technology has been used in abstention of antigen-specific peptides with high specificity and affinity for development of bioassays for the identification of various biomarkers [72]. Phage-based assays have been developed for detect *M. tuberculosis* in clinical samples and culture, as well as for to identify resistance to anti-tubercular drug rifampicin. Currently these assays are commercially available as FAST Plaque-TB™ and Phage Tek MB™ kits [73-76]. Until now, these assays require more development for to enhance the interpretation of the results and minimize errors [77]. In this way has been proposed a phage-based bioassay that involves magnetoelastic elastic biosensors with the end to obtain a miniaturized device capable of detecting multiple agents [78].

Whole-cell biosensor

Contrary to sensors that use purified cellular components. Whole cell biosensor are a choice for avoid the purification costs, in addition these sensors are easier to handle and are more stable in environments and can increase their sensitivity by the use of reporter genes [79]. Between them luciferase, which produces a light emitting reaction, is as commonly used enzyme for whole-cell biosensors, that can be employed for detect bacterial contamination. In this case bacteria expressing the luciferase operon have been used to detect antimicrobials that affect the transcriptional/translational machinery [80,81]. In addition microbes have the ability of metabolize a large number of chemical compounds in different conditions making them an important alternative for field data [82,83]. Also, these technologies in combination with micro cantilever arrays using Ink-jet device can be useful for perform microbial monitoring [84]. Equally a biosensor consisting of vibrating cantilevers with bacteria fixed have shown the ability of calculate microbial mass within 1 h, as well as to assess antimicrobial activity on *P. aeruginosa* of antibiotics vancomycin and colistin [85].

Otherwise, whole cell biosensors can be useful in discover new drugs with diverse mechanisms of action, in that way a *Bacillus subtilis* biosensors have been used for study antibacterial activity of anti-infective, looking for RNA polymerase inhibitors and DNA intercalators [86].

Biofilm biosensor

Biofilm biosensors are an approach of whole-cell living biosensors for the development of bioreporters useful in environments monitoring and drug discovery [87]. In this order of ideas biofilm biosensors with oxygen electrode have been developed for to measure the respiration rate of microorganisms present in a water purification system. But is important to know that these biosensors need the constant care of viable cells and the expense of nutrients if prolonged storage is required [88]. This Electro-Active Biofilm (EAB) has the quality of conductance to a direct electrochemical connection without mediators. Is necessary to study this electrical capability of microorganisms under this form and their applications as electrochemical biosensors to monitor the development of biofilms and compounds [89]. Other interesting approach is the development of Microbial Fuel Cell (MFC) biofilm biosensor, designed for the monitoring and control of anaerobic digestion, with the capability of offer results about microbial growth in anaerobic systems [90]. Bacterial communication known as Quorum Sensing (QS) is mediated by N-Acyl-Homoserine Lactones (AHL) under regulation of LuxR-OHHL gene transcription [91]. In this way is possible to develop bacterial biosensors with the ability to detect the production of AHLs. These biosensors contain a functional LuxR-family protein, which positively regulates the transcription of a reporter gene. AHL biosensor strains can be used for establish the behavior of microbial cell in different conditions and the possibility of biofilm formation [92,93].

Fluorescent biosensor

In the development of fluorescent microbial biosensors Green Fluorescent Protein (GFP) is most commonly used due to their stability [94,95]. In this sense, recombinant *E. coli* that express GFP was used as a screening platform to analyze antimicrobial

activity of silver nanoparticles (AgNPs), in where cell lysis in AgNp treated microorganisms was demonstrated by the increase of GFP fluorescence [96]. Equally GFP fluorescence is useful in antifungal drug discovery employing a transformed strain of *Aureobasidium pullulans* whose results are liable to be quantified by using fluorescence spectrophotometry measuring the direct relationship between fluorescence and the number of viable spores [97]. The advantage of GFP based biosensors are their ease of construction with conventional molecular biology techniques [98]. But is important take in consideration that the transformation with GFP can affect the physiology of bacterial cells and this can affect the accuracy of data obtained under this method [99]. On the other hand antimicrobial peptides as cecropin P1 can be fluorescently labeled with Cy5 dye for replacement of labeled antibodies, the basis of this protocol is to use the affinity of the peptides to the lipopolysaccharide component of bacterial cell walls, and the strength of this binding can increase the optical signal and the sensitivity. Being 10 times more sensitive in detecting *E. coli* that an antibody biosensor [100].

Nanosensor

One interesting application of nanotechnology is in the development of biosensors, the use of nanosensors is an important tool with the ability of obtain the information from nanoparticles, being classified in physical, chemical and biological nanosensors [101]. These nanosensors have the great advantage of can be inserted in nanowires, which are nanostructures with important properties (mechanical, electrical, thermal and multifunctional), providing a greatly increased sensitivity and specificity of electrochemical sensors [26]. Due to the emergence of MDR bacteria, are necessary design platforms that use this technology to improve the accuracy and sensitivity of AST methods. An approach in this area is the use of super-paramagnetic iron oxide nanoparticles as AST nanosensors through magnetic relaxation. This method has the ability of quantify polysaccharides, as well as measure the metabolic activity and obtain MIC values in blood [102]. Equally dextran-coated gold nanoparticles can be used in AST assays based on the concanavalin A-induced clustering in presence or absence of microbial growth. This gold AST nanoparticle-based method offer results within 3 hours and can be adapted in HTS platforms [103,104].

In addition, using nanotechnological cantilever assays is possible study the interaction between MDR bacteria and antibiotics (Figure 3) [105]. The cantilever sensor acts as a transducer between bacterial cell wall and antibiotic, this method present high sensitivity and specificity. Also, have the capability to detect the drug target interactions using a laser, due to the disruptions of wall can be measured in real time with nano-scale precision [106].

Microfluidics Biosensing

The use of microfluidics platforms for AST methods has been evidenced in models employing bacteria with standard susceptibility patterns. Wherein this method was able to provide a result within two hours, which facilitates the diagnosis at the point of care [107]. In this way an AST assay using gas permeable micro channels with similar dimensions to that of a microbial cell has been developed, determining cellular lysis by AC electrokinetic technique, this protocol in an antimicrobial model with urinary pathogens was able to determinate susceptibility patterns in less than one hour [108]. Other methods use a Microfluidic Agarose Channel (MAC) system, determining MICs by evaluation of cell growth under microscopic observation in 3-4 hours [109]. Equally the micro channels in a microfluidic platform can be revealed using measurements of fluorescence intensity [110], or can be used for study of antimicrobial resistance induced by mechanical stress [111], which are advantages of the versatility of this platform including the decrease in evaluation time, the increased sensitivity of detection, the decrease in the number of reagents to be used [110], and the possibility to be included in a chip-based system [112].

On the other hand the combination of microfluidics with optical systems in optofluidic biosensors has been used in the detection of viruses and bacteria, with the advantage to differentiate in multiplex platform virus particles including Vaccinia and Ebola, as well as MRSA in a fast and automated technique useful in epidemiological surveillance [113,114].

Antimicrobial Drug Prospecting

Other important use of biosensors is in the implementation of bioprospecting devices for detection of antibacterial (Table 1). In this mode have been used two main methods for the recognition of antimicrobials. The first one employs immobilized aptamers

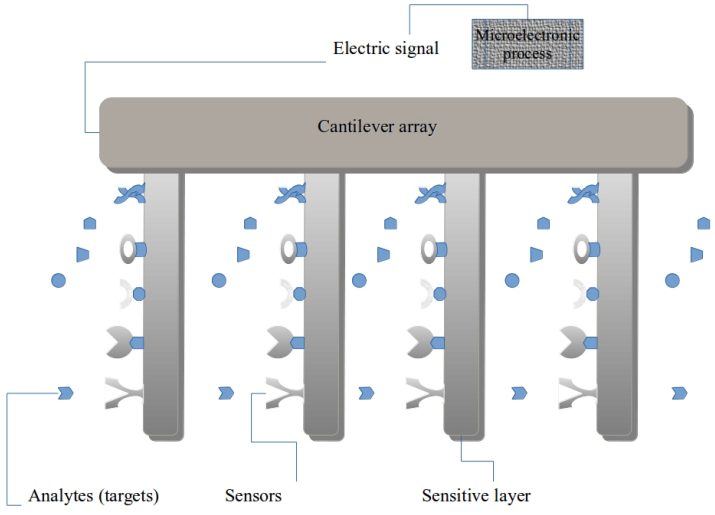

Figure 3: Cantilever array for biosensor development.

(aptasensors) that are oligonucleic acids that interacts with the analyte of interest (protein, toxin) producing detectable signals. The second way for antimicrobial identification is the use of antibody biosensors [8,115]. Aptamers biosensors can be produced by SELEX process that select nucleic acid ligands specific of protein target [116]. In this sense RNA-aptasensor for detection of neomycin B with high selectivity has been developed, making it an interesting platform to identify aminoglycosides [117]. In addition, this aptamer-based biosensor can be adapted in a cantilever array which increases the sensitivity and specificity of the device for detects the antibiotic oxytetracycline [118]. Also, the use of aptamers presents various advantages in comparison with antibodies as their small size, chemical stability and cost; as well as structural versatility, thus may develop many different new biosensors with higher sensitivity and specificity [119,120].

Cells containing reporters have been used in screening for novel drug candidates and in the detection of bioactive compounds in environmental samples. *Bacillus subtilis* with luciferase reporter gene is useful in detects compounds that inhibit biosynthetic pathways of bacteria and are compatible with high-throughput screening. This anti-*Bacillus subtilis* platform was evaluated against 14,000 natural products with the capability of detects a new action mechanism of the antibiotic ferrimycin A1 [7]. Also this method can monitor metabolite production, as mevalonate that is present in the isoprenoid biosynthesis and can be identified using GFP as reporter, with the end of detect environmental strains with potential of metabolite synthesis [121,122].

On the other hand lipid A-based affinity biosensor technology is a tool developed for to assess natural products with the ability of neutralizes or destroy LPS (lipopolysaccharide), a component of Gram-negative bacteria. This method was proved with 78 plant extracts from Chinese herbs, identifying to the medicinal plant *Paeonia suffruticosa* as the more potent anti-LPS extract. These results showed the potential of biosensors in bioprospecting programs looking for antimicrobial drugs from natural sources [123].

Conclusions

Biosensors, are an important tool in drug discovery, and can be useful both in the screening process as in bioprospecting evaluation [124]. For apply a biosensor screening platform is necessary take in account the following parameters: specificity, kinetics, affinity and concentration of analyte for detect [125]. Also is very important the selection of transducer (optical, electrochemical, acoustic) depending of their applications (portable device in field, research laboratory, clinical practice), for develop a biosensor for clinical area is of importance to comply with these characteristics: portability, rapidity, and cost-effectiveness [10,28]. In this way the development of portable devices that use smaller sample volume is necessary to carry out field tests with greater agility and speed, in where electrochemical devices have the ability in a low cost of to be miniaturized to increase portability with high sensitivity and specificity [126]. Equally, biosensors as powerful bioanalytical technology require before implementation for in vitro diagnostics to be evaluated in two components as are analytical verification and clinical validation. With the end to determinate their accuracy, precision, analytical sensitivity, analytical specificity, cross-reactivity, interference, sample matrix effects, clinical accuracy, and predictive positive/negative values with prevalence; in order to establish the advantages and disadvantages of using this technology in field. This evaluation necessitates a multidisciplinary approach for to be developed of analytical scientists, test developers, clinicians, and regulatory agencies [127].

Equally, nanomaterials have a promising impact in biosensors development by their broad possibilities in manufacturing for obtain electrochemical bioassays [128,129], as well as built nanostructures that detect a particular pathogen and determine if drug-resistant [130]. On the other hand, the combinations of biosensors with microfluidics technology have the capability of development of new AST methods at the point of care [131]. Due to microfluidic possess the ability to integrate biosensor with microscopical visualization for obtain automated images. Also, microfluidic devices can perform isolation, purification and manipulation of clinical samples, as well as fix nanoparticles, biomolecules, bacteriophages, and cells in drug discovery and diagnostic models [132,133]. Likewise, AST development using microfluidics devices is possible, because have the ability of quantify antibiotic effects, enhancing sensitivity and specificity [110]. In this way an interesting approach uses microbead-based microfluidic devices for to improve detection efficiency due to the increased volume of surface immobilization [134].

Finally, the major problem in implementation of biosensors in point of care is the sample preparation; many technologies have been evaluated using isolated microorganisms without determining the matrix effect of clinical samples on device [135]. For that reason, the development of new multiplexing biosensors with multi-array in screening platforms as optofluidics and various biomarkers may constitute an advance in solving this problem [136,137].

References

1. Leung E, Weil DE, Raviglione M, Nakatani H; World Health Organization World Health Day Antimicrobial Resistance Technical Working Group (2011) The WHO policy package to combat antimicrobial resistance. Bull World Health Organ 89: 390-392.
2. Infectious Diseases Society of America (IDSA), Spellberg B, Blaser M, Guidos RJ, Boucher HW, et al. (2011) Combating antimicrobial resistance: policy recommendations to save lives. Clin Infect Dis 52 Suppl 5: S397-428.
3. Bassetti M, Merelli M, Temperoni C, Astilean A (2013) New antibiotics for bad bugs: where are we? Ann Clin Microbiol Antimicrob 12: 22.
4. Infectious Diseases Society of America1 (2010) The 10 x '20 Initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. Clin Infect Dis 50: 1081-1083.
5. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, et al. (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48: 1-12.
6. Boucher HW, Talbot GH, Benjamin DK Jr, Bradley J, Guidos RJ, et al. (2013) 10 x '20 Progress--development of new drugs active against gram-negative bacilli: an update from the Infectious Diseases Society of America. Clin Infect Dis 56: 1685-1694.
7. Urban A, Eckermann S, Fast B, Metzger S, Gehling M, et al. (2007) Novel whole-cell antibiotic biosensors for compound discovery. Appl Environ Microbiol 73: 6436-6443.
8. Reder-Christ K, Bendas G (2011) Biosensor applications in the field of antibiotic research--a review of recent developments. Sensors (Basel) 11: 9450-9466.
9. Finch R, Hunter PA (2006) Antibiotic resistance--action to promote new technologies: report of an EU Intergovernmental Conference held in Birmingham, UK, 12-13 December 2005. J Antimicrob Chemother 58 Suppl 1: i3-3i22.
10. Mach KE, Mohan R, Baron EJ, Shih MC, Gau V, et al. (2011) A biosensor platform for rapid antimicrobial susceptibility testing directly from clinical samples. J Urol 185: 148-153.
11. Saleem M (2013) Biosensors a promising future in measurements. IOP Conference Series: Materials Science and Engineering 51: 012012.
12. McGrath TF, Elliott CT, Fodey TL (2012) Biosensors for the analysis of microbiological and chemical contaminants in food. Anal Bioanal Chem 403: 75-92.

13. Murugaiyan SB, Ramasamy R, Gopal N, Kuzhandaivelu V (2014) Biosensors in clinical chemistry: An overview. Adv Biomed Res 3: 67.

14. Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C (2010) An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnol Adv 28: 232-254.

15. van Belkum A, Dunne WM Jr (2013) Next-generation antimicrobial susceptibility testing. J Clin Microbiol 51: 2018-2024.

16. Bissonnette L, Bergeron MG (2010) Diagnosing infections--current and anticipated technologies for point-of-care diagnostics and home-based testing. Clin Microbiol Infect 16: 1044-1053.

17. Llandro J, Palfreyman JJ, Ionescu A, Barnes CH (2010) Magnetic biosensor technologies for medical applications: a review. Med Biol Eng Comput 48: 977-998.

18. Kinnunen P, Sinn I, McNaughton BH, Newton DW, Burns MA, et al. (2011) Monitoring the growth and drug susceptibility of individual bacteria using asynchronous magnetic bead rotation sensors. Biosens Bioelectron 26: 2751-2755.

19. Sinn I, Kinnunen P, Albertson T, McNaughton BH, Newton DW, et al. (2011) Asynchronous magnetic bead rotation (AMBR) biosensor in microfluidic droplets for rapid bacterial growth and susceptibility measurements. Lab Chip 11: 2604-2611.

20. Kinnunen P, McNaughton BH, Albertson T, Sinn I, Mofakham S, et al. (2012) Self-assembled magnetic bead biosensor for measuring bacterial growth and antimicrobial susceptibility testing. Small 8: 2477-2482.

21. Sinn I, Albertson T, Kinnunen P, Breslauer DN, McNaughton BH, et al. (2012) Asynchronous magnetic bead rotation microviscometer for rapid, sensitive, and label-free studies of bacterial growth and drug sensitivity. Anal Chem 84: 5250-5256.

22. Wan J, Shu H, Huang S, Fiebor B, Chen I et al. (2007) Phage-based magnetoelastic wireless biosensors for detecting *Bacillus* anthracis spores. Sensors Journal, IEEE 7: 470-477.

23. Veloso A, Cheng X, Kerman K (2012) 1-electrochemical biosensors for medical applications. In: Higson S, ed. Biosensors for medical applications. Woodhead Publishing. pp. 3-40.

24. Grieshaber D, MacKenzie R, Voeroes J, Reimhult E (2008) Electrochemical biosensors-Sensor principles and architectures. Sensors 8: 1400-1458.

25. Renedo OD, Alonso-Lomillo MA, Martínez MJ (2007) Recent developments in the field of screen-printed electrodes and their related applications. Talanta 73: 202-219.

26. Yogeswaran U, Chen S (2008) A review on the electrochemical sensors and biosensors composed of nanowires as sensing material. Sensors 8: 290-313.

27. Daly R, Kumar S, Lukacs G, Lee K, Weidlich A et al. (2012) Cell Proliferation Tracking Using Graphene Sensor Arrays. Journal of Sensors 2012: 7.

28. El-Ali J, Sorger PK, Jensen KF (2006) Cells on chips. Nature 442: 403-411.

29. Neumann H, Neumann-Staubitz P (2010) Synthetic biology approaches in drug discovery and pharmaceutical biotechnology. Appl Microbiol Biotechnol 87: 75-86.

30. Mannoor MS, Zhang S, Link AJ, McAlpine MC (2010) Electrical detection of pathogenic bacteria via immobilized antimicrobial peptides. Proc Natl Acad Sci USA 107: 19207-19212.

31. Mach KE, Wong PK, Liao JC (2011) Biosensor diagnosis of urinary tract infections: a path to better treatment? Trends Pharmacol Sci 32: 330-336.

32. Mach KE, Du CB, Phull H, Haake DA, Shih MC, et al. (2009) Multiplex pathogen identification for polymicrobial urinary tract infections using biosensor technology: a prospective clinical study. J Urol 182: 2735-2741.

33. Mohan R, Mach KE, Bercovici M, Pan Y, Dhulipala L, et al. (2011) Clinical validation of integrated nucleic acid and protein detection on an electrochemical biosensor array for urinary tract infection diagnosis. PLoS One 6: e26846.

34. Sin ML, Liu T, Pyne JD, Gau V, Liao JC, et al. (2012) In situ electrokinetic enhancement for self-assembled-monolayer-based electrochemical biosensing. Anal Chem 84: 2702-2707.

35. Halford C, Gonzalez R, Campuzano S, Hu B, Babbitt JT, et al. (2013) Rapid antimicrobial susceptibility testing by sensitive detection of precursor rRNA using a novel electrochemical biosensing platform. Antimicrob Agents Chemother 57: 936-943.

36. Labib M, Zamay AS, Kolovskaya OS, Reshetneva IT, Zamay GS, et al. (2012) Aptamer-based viability impedimetric sensor for bacteria. Anal Chem 84: 8966-8969.

37. Labib M, Zamay AS, Muharemagic D, Chechik AV, Bell JC, et al. (2012) Aptamer-based viability impedimetric sensor for viruses. Anal Chem 84: 1813-1816.

38. Chung CC, Cheng IF, Yang WH, Chang HC (2011) Antibiotic susceptibility test based on the dielectrophoretic behavior of elongated Escherichia coli with cephalexin treatment. Biomicrofluidics 5: 21102.

39. Puttaswamy s, Lee b, Amighi b, Chakraborty s, Sengupta s (2011) Novel electrical method for the rapid determination of minimum inhibitory concentration (MIC) and assay of bactericidal/bacteriostatic activity. J Biosens Bioelectron S2: 003.

40. Motaweh HHA, Ali FM, El-Khattib AM, Sabry SA, Abo-Neima SE (2014). Control of *Staphylococcus aureus* activity in rats using electromagnetic signals at resonance frequency" in vivo study. ESJ 10: 462-472.

41. Zibaii MI, Kazemi A, Latifi H, Azar MK, Hosseini SM, et al. (2010) Measuring bacterial growth by refractive index tapered fiber optic biosensor. J Photochem Photobiol B 101: 313-320.

42. Geschwindner S, Carlsson JF, Knecht W (2012) Application of optical biosensors in small-molecule screening activities. Sensors (Basel) 12: 4311-4323.

43. Ligler FS (2009) Perspective on optical biosensors and integrated sensor systems. Anal Chem 81: 519-526.

44. Taylor AD, Ladd J, Homola J, Jiang S (2008) Surface plasmon resonance (spr) sensors for the detection of bacterial pathogens. In: Zourob M, Elwary S, Turner A, eds. Principles of bacterial detection: biosensors, recognition receptors and microsystems. Springer. pp. 83-108.

45. Mozsolits H, Wirth HJ, Werkmeister J, Aguilar MI (2001) Analysis of antimicrobial peptide interactions with hybrid bilayer membrane systems using surface plasmon resonance. Biochim Biophys Acta 1512: 64-76.

46. Besenicar M, Macek P, Lakey JH, Anderluh G (2006) Surface plasmon resonance in protein-membrane interactions. Chem Phys Lipids 141: 169-178.

47. Wang Y, Ye Z, Si C, Ying Y (2011) Subtractive inhibition assay for the detection of E. coli O157:H7 using surface plasmon resonance. Sensors (Basel) 11: 2728-2739.

48. Chiang YL, Lin CH, Yen MY, Su YD, Chen SJ, et al. (2009) Innovative antimicrobial susceptibility testing method using surface plasmon resonance. Biosens Bioelectron 24: 1905-1910.

49. Arlett JL, Myers EB, Roukes ML (2011) Comparative advantages of mechanical biosensors. Nat Nanotechnol 6: 203-215.

50. Daghestani HN, Day BW (2010) Theory and applications of surface plasmon resonance, resonant mirror, resonant waveguide grating, and dual polarization interferometry biosensors. Sensors (Basel) 10: 9630-9646.

51. Mehrvar M, Bis C, Scharer JM, Moo-Young M, Luong JH (2000) Fiber-optic biosensors-trends and advances. Analytical sciences 16: 677-692.

52. Bosch ME, Sánchez AJR, Rojas FS, Ojeda CB (2007) Recent development in optical fiber biosensors. Sensors 7: 797-859.

53. Rocha-Gaso MI, March-Iborra C, Montoya-Baides A, Arnau-Vives A (2009) Surface generated acoustic wave biosensors for the detection of pathogens: a review. Sensors (Basel) 9: 5740-5769.

54. Tan H, Le D, Li J, Wei W, Yao S (1998) A rapid method for determination of in vitro susceptibility to antibiotics with a bulk acoustic wave bacterial growth biosensor. Lett Appl Microbiol 27: 57-61.

55. Jelinek R, Silbert L (2009) Biomimetic approaches for studying membrane processes. Mol Biosyst 5: 811-818.

56. Senyürek I, Paulmann M, Sinnberg T, Kalbacher H, Deeg M, et al. (2009) Dermcidin-derived peptides show a different mode of action than the cathelicidin LL-37 against *Staphylococcus aureus*. Antimicrob Agents Chemother 53: 2499-2509.

57. Al-Kaddah S, Reder-Christ K, Klocek G, Wiedemann I, Brunschweiger M, et al. (2010) Analysis of membrane interactions of antibiotic peptides using ITC and biosensor measurements. Biophys Chem 152: 145-152.

58. Chang K, Chang C, Chen C (2007) A surface acoustic wave sensor modified from a wireless transmitter for the monitoring of the growth of bacteria. Sens Actuators B Chem 125: 207-213.

59. Omidfar K, Khorsand F, Darziani Azizi M (2013) New analytical applications of gold nanoparticles as label in antibody based sensors. Biosens Bioelectron 43: 336-347.

60. Dover JE, Hwang GM, Mullen EH, Prorok BC, Suh SJ (2009) Recent advances in peptide probe-based biosensors for detection of infectious agents. J Microbiol Methods 78: 10-19.

61. Nugaeva N, Gfeller KY, Backmann N, Düggelin M, Lang HP, et al. (2007) An antibody-sensitized microfabricated cantilever for the growth detection of *Aspergillus niger* spores. Microsc Microanal 13: 13-17.

62. Kadlec MW, You D, Liao JC, Wong PK (2013) A Cell Phone-Based Microphotometric System for Rapid Antimicrobial Susceptibility Testing. J Lab Autom 19: 258-266.

63. Zhao W, Ali MM, Aguirre SD, Brook MA, Li Y (2008) Paper-based bioassays using gold nanoparticle colorimetric probes. Anal Chem 80: 8431-8437.

64. Miranda OR, Li X, Garcia-Gonzalez L, Zhu ZJ, Yan B, et al. (2011) Colorimetric bacteria sensing using a supramolecular enzyme-nanoparticle biosensor. J Am Chem Soc 133: 9650-9653.

65. Rogers KR (2006) Recent advances in biosensor techniques for environmental monitoring. Anal Chim Acta 568: 222-231.

66. Jordana-Lluch E, Giménez M1, Quesada MD1, Ausina V2, Martró E3 (2014) Improving the diagnosis of bloodstream infections: PCR coupled with mass spectrometry. Biomed Res Int 2014: 501214.

67. Ecker DJ, Sampath R, Massire C, Blyn LB, Hall TA, et al. (2008) Ibis T5000: a universal biosensor approach for microbiology. Nat Rev Microbiol 6: 553-558.

68. Wolk DM, Kaleta EJ, Wysocki VH (2012) PCR-electrospray ionization mass spectrometry: the potential to change infectious disease diagnostics in clinical and public health laboratories. J Mol Diagn 14: 295-304.

69. Lee SC, Wu MS, Shih HJ, Huang SH, Chiou MJ, et al. (2013) Identification of vancomycin-resistant enterococci clones and inter-hospital spread during an outbreak in Taiwan. BMC Infect Dis 13: 163.

70. Gooding JJ (2006) Biosensor technology for detecting biological warfare agents: Recent progress and future trends. Anal Chim Acta 559: 137-151.

71. Brinkman CL, Vergidis P, Uhl JR, Pritt BS, Cockerill FR, et al. (2013) PCR-electrospray ionization mass spectrometry for direct detection of pathogens and antimicrobial resistance from heart valves in patients with infective endocarditis. J Clin Microbiol 51: 2040-2046.

72. Lee JW, Song J, Hwang MP, Lee KH (2013) Nanoscale bacteriophage biosensors beyond phage display. Int J Nanomedicine 8: 3917-3925.

73. Pai M, Kalantri S, Pascopella L, Riley LW, Reingold AL (2005) Bacteriophage-based assays for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a meta-analysis. J Infect 51: 175-187.

74. Piuri M, Jacobs WR Jr, Hatfull GF (2009) Fluoromycobacteriophages for rapid, specific, and sensitive antibiotic susceptibility testing of *Mycobacterium tuberculosis*. PLoS One 4: e4870.

75. Jacobs WR Jr, Barletta RG, Udani R, Chan J, Kalkut G, et al. (1993) Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. Science 260: 819-822.

76. Carrière C, Riska PF, Zimhony O, Kriakov J, Bardarov S, et al. (1997) Conditionally replicating luciferase reporter phages: improved sensitivity for rapid detection and assessment of drug susceptibility of *Mycobacterium tuberculosis*. J Clin Microbiol 35: 3232-3239.

77. Minion J, Pai M (2010) Bacteriophage assays for rifampicin resistance detection in *Mycobacterium tuberculosis*: updated meta-analysis. Int J Tuberc Lung Dis 14: 941-951.

78. Grimes CA, Roy SC, Rani S, Cai Q (2011) Theory, instrumentation and applications of magnetoelastic resonance sensors: a review. Sensors (Basel) 11: 2809-2844.

79. Park M, Tsai SL, Chen W (2013) Microbial biosensors: engineered microorganisms as the sensing machinery. Sensors (Basel) 13: 5777-5795.

80. Galluzzi L, Karp M (2006) Whole cell strategies based on lux genes for high throughput applications toward new antimicrobials. Comb Chem High Throughput Screen 9: 501-514.

81. Ankö ML, Kurittu J, Karp M (2002) An *Escherichia coli* biosensor strain for amplified and high throughput detection of antimicrobial agents. J Biomol Screen 7: 119-125.

82. D'Souza SF (2001) Microbial biosensors. Biosens Bioelectron 16: 337-353.

83. Lukacs G, Maloney N, Hegner M (2012) Ink-Jet Printing: Perfect Tool for Cantilever Array Sensor Preparation for Microbial Growth Detection. Journal of Sensors 2012: 7.

84. Lei Y, Chen W, Mulchandani A (2006) Microbial biosensors. Anal Chim Acta 568: 200-210.

85. Gfeller KY, Nugaeva N, Hegner M (2005) Rapid biosensor for detection of antibiotic-selective growth of *Escherichia coli*. Appl Environ Microbiol 71: 2626-2631.

86. Mariner KR, Ooi N, Roebuck D, O'Neill AJ, Chopra I (2011) Further characterization of *Bacillus subtilis* antibiotic biosensors and their use for antibacterial mode-of-action studies. Antimicrob Agents Chemother 55: 1784-1786.

87. Harms H, Wells MC, van der Meer JR (2006) Whole-cell living biosensors--are they ready for environmental application? Appl Microbiol Biotechnol 70: 273-280.

88. Ponamoreva ON, Arliapov VA, Alferov VA, Reshetilov AN (2011) Microbial biosensors for detection of biological oxygen demand (a review). Prikl Biokhim Mikrobiol 47: 5-15.

89. Erable B, Duțeanu NM, Ghangrekar MM, Dumas C, Scott K (2010) Application of electro-active biofilms. Biofouling 26: 57-71.

90. Liu Z, Liu J, Zhang S, Xing XH, Su Z (2011) Microbial fuel cell based biosensor for in situ monitoring of anaerobic digestion process. Bioresour Technol 102: 10221-10229.

91. Bueno J (2014) Anti-Biofilm Drug Susceptibility Testing Methods: Looking for New Strategies against Resistance Mechanism. J Microbial Biochem Technol S3: 004.

92. Steindler L, Venturi V (2007) Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266: 1-9.

93. Fletcher M, Cámara M, Barrett DA, Williams P (2014) Biosensors for qualitative and semiquantitative analysis of quorum sensing signal molecules. Methods Mol Biol 1149: 245-254.

94. Sánchez JG, Kouznetsov VV (2010) Antimycobacterial susceptibility testing methods for natural products research. Braz J Microbiol 41: 270-277.

95. Dai C, Choi S (2013) Technology and applications of microbial biosensor. OJAB 2: 83.

96. Gogoi SK, Gopinath P, Paul A, Ramesh A, Ghosh SS, et al. (2006) Green fluorescent protein-expressing Escherichia coli as a model system for investigating the antimicrobial activities of silver nanoparticles. Langmuir 22: 9322-9328.

97. Webb JS, Barratt SR, Sabev H, Nixon M, Eastwood IM, et al. (2001) Green fluorescent protein as a novel indicator of antimicrobial susceptibility in *Aureobasidium pullulans*. Appl Environ Microbiol 67: 5614-5620.

98. Ibraheem A, Campbell RE (2010) Designs and applications of fluorescent protein-based biosensors. Curr Opin Chem Biol 14: 30-36.

99. Allison DG, Sattenstall MA (2007) The influence of green fluorescent protein incorporation on bacterial physiology: a note of caution. J Appl Microbiol 103: 318-324.

100. Arcidiacono S, Pivarnik P, Mello CM, Senecal A (2008) Cy5 labeled antimicrobial peptides for enhanced detection of *Escherichia coli* O157:H7. Biosens Bioelectron 23: 1721-1727.

101. Usibe BE, Menkiti AI, Onuu MU, Ogbulezie JC (2013) Development and analysis of a potential nanosensor communication network using carbon nanotubes. IJMSE 3: 4-10.

102. Kaittanis C, Nath S, Perez JM (2008) Rapid nanoparticle-mediated monitoring

of bacterial metabolic activity and assessment of antimicrobial susceptibility in blood with magnetic relaxation. PLoS One 3: e3253.

103. Ndieyira JW, Watari M, Barrera AD, Zhou D, Vögtli M, et al. (2008) Nanomechanical detection of antibiotic-mucopeptide binding in a model for superbug drug resistance. Nat Nanotechnol 3: 691-696.

104. Nath S, Kaittanis C, Tinkham A, Perez JM (2008) Dextran-coated gold nanoparticles for the assessment of antimicrobial susceptibility. Anal Chem 80: 1033-1038.

105. Muralimohan A, Eun YJ, Bhattacharyya B, Weibel DB (2009) Dissecting microbiological systems using materials science. Trends Microbiol 17: 100-108.

106. Ndieyira JW, Watari M, McKendry RA (2013) Nanomechanics of drug-target interactions and antibacterial resistance detection. J Vis Exp : e50719.

107. Chen CH, Lu Y, Sin ML, Mach KE, Zhang DD, et al. (2010) Antimicrobial susceptibility testing using high surface-to-volume ratio microchannels. Anal Chem 82: 1012-1019.

108. Lu Y, Gao J, Zhang DD, Gau V, Liao JC, et al. (2013) Single cell antimicrobial susceptibility testing by confined microchannels and electrokinetic loading. Anal Chem 85: 3971-3976.

109. Choi J, Jung YG, Kim J, Kim S, Jung Y, et al. (2013) Rapid antibiotic susceptibility testing by tracking single cell growth in a microfluidic agarose channel system. Lab Chip 13: 280-287.

110. Mohan R, Mukherjee A, Sevgen SE, Sanpitakseree C, Lee J, et al. (2013) A multiplexed microfluidic platform for rapid antibiotic susceptibility testing. Biosens Bioelectron 49: 118-125.

111. Kalashnikov M, Lee JC, Campbell J, Sharon A, Sauer-Budge AF (2012) A microfluidic platform for rapid, stress-induced antibiotic susceptibility testing of Staphylococcus aureus. Lab Chip 12: 4523-4532.

112. Li B, Qiu Y, Glidle A, McIlvenna D, Luo Q, et al. (2014) Gradient microfluidics enables rapid bacterial growth inhibition testing. Anal Chem 86: 3131-3137.

113. Yanik AA, Huang M, Kamohara O, Artar A, Geisbert TW, et al. (2010) An optofluidic nanoplasmonic biosensor for direct detection of live viruses from biological media. Nano Lett 10: 4962-4969.

114. Lu X, Samuelson DR, Xu Y, Zhang H, Wang S, et al. (2013) Detecting and tracking nosocomial methicillin-resistant *Staphylococcus aureus* using a microfluidic SERS biosensor. Anal Chem 85: 2320-2327.

115. Rodriguez MC, Kawde AN, Wang J (2005) Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of the surface charge. Chem Commun (Camb): 4267-4269.

116. Tombelli S, Mascini M (2010) Aptamers biosensors for pharmaceutical compounds. Comb Chem High Throughput Screen 13: 641-649.

117. de-los-Santos-Alvarez N1, Lobo-Castañón MJ, Miranda-Ordieres AJ, Tuñón-Blanco P (2007) Modified-RNA aptamer-based sensor for competitive impedimetric assay of neomycin B. J Am Chem Soc 129: 3808-3809.

118. Hou H, Bai X, Xing C, Gu N, Zhang B, et al. (2013) Aptamer-based cantilever array sensors for oxytetracycline detection. Anal Chem 85: 2010-2014.

119. Song S, Wang L, Li J, Fan C, Zhao J (2008) Aptamer-based biosensors. Trends Analyt Chem 27: 108-117.

120. Song KM, Lee S, Ban C (2012) Aptamers and their biological applications. Sensors (Basel) 12: 612-631.

121. Pfleger BF, Pitera DJ, Newman JD, Martin VJ, Keasling JD (2007) Microbial sensors for small molecules: development of a mevalonate biosensor. Metab Eng 9: 30-38.

122. Zhang F, Keasling J (2011) Biosensors and their applications in microbial metabolic engineering. Trends Microbiol 19: 323-329.

123. Yao J, Chen Y, Wang N, Jiang D, Zheng J (2014) Lipid A-based affinity biosensor for screening anti-sepsis components from herbs. Biosci Rep 34.

124. Gonçalves AM, Pedro AQ, Santos FM, Martins LM, Maia CJ, et al. (2014) Trends in protein-based biosensor assemblies for drug screening and pharmaceutical kinetic studies. Molecules 19: 12461-12485.

125. Indyk HE (2006) Optical biosensors: making sense of interactions. Chemistry in New Zealand 70: 42.

126. Hayat A, Marty JL (2014) Disposable screen printed electrochemical sensors: tools for environmental monitoring. Sensors (Basel) 14: 10432-10453.

127. Sergeev NV, Herold KE, Rasooly A (2008) Regulatory and validation issues for biosensors and related bioanalytical technologies. In: Marks RS, Cullen DC, Karube I, Lowe CR, Weetall HH, eds. Handbook of biosensors and biochips. John Wiley and Sons, Ltd. pp 1289-1302.

128. Pumera M, Sanchez S, Ichinose I, Tang J (2007) Electrochemical nanobiosensors. Sensors and Actuators B: Chemical 123: 1195-1205.

129. Yen P, Lu Y, Lin C, Hwang C, Andrew Yeh J et al. (2014) Emerging Electrical Biosensors for Detecting Pathogens and Antimicrobial Susceptibility Tests. Current Organic Chemistry 18: 165-172

130. Kaittanis C, Santra S, Perez JM (2010) Emerging nanotechnology-based strategies for the identification of microbial pathogenesis. Adv Drug Deliv Rev 62: 408-423.

131. Chen CH, Lu Y, Sin ML, Mach KE, Zhang DD, et al. (2010) Antimicrobial susceptibility testing using high surface-to-volume ratio microchannels. Anal Chem 82: 1012-1019.

132. Neuži P, Giselbrecht S, Länge K, Huang TJ, Manz A (2012) Revisiting lab-on-a-chip technology for drug discovery. Nat Rev Drug Discov 11: 620-632.

133. Mark D, Haeberle S, Roth G, von Stetten F, Zengerle R (2010) Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. Chem Soc Rev 39: 1153-1182.

134. Yoo JH, Woo DH, Chang M, Chun M (2014) Microfluidic based biosensing for Escherichia coli detection by embedding antimicrobial peptide-labeled beads. Sens Actuators B Chem 191: 211-218.

135. Sin ML, Mach KE, Wong PK, Liao JC (2014) Advances and challenges in biosensor-based diagnosis of infectious diseases. Expert Rev Mol Diagn 14: 225-244.

136. Erickson D, Mandal S, Yang AH, Cordovez B (2008) Nanobiosensors: optofluidic, electrical and mechanical approaches to biomolecular detection at the nanoscale. Microfluid Nanofluidics 4: 33-52.

137. Cetin AE, Coskun AF, Galarreta BC, Huang M, Herman D et al. (2014) Handheld high-throughput plasmonic biosensor using computational on-chip imaging. Light: Science and Applications 3: e122.

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