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Research review paper

Antimicrobial peptides: Promising alternatives over conventional capture ligands for biosensor-based detection of pathogenic bacteria

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ABSTRACT

The detection of pathogenic bacteria using biosensing techniques could be a potential alternative to traditional culture based methods. However, the low specificity and sensitivity of conventional biosensors, critically related to the choice of bio-recognition elements, limit their practical applicability. Mammalian antibodies have been widely investigated as biorecognition ligands due to high specificity and technological advancement in antibody production. However, antibody-based biosensors are not considered as an efficient approach due to the batch-to-batch inconsistencies as well as low stability. In recent years, antimicrobial peptides (AMPs) have been increasingly investigated as ligands as they have demonstrated high stability and possessed multiple sites for capturing bacteria. The conjugation of chemo-selective groups with AMPs has allowed effective immobilization of peptides on biosensor surface. However, the specificity of AMPs is a major concern for consideration as an efficient ligand. In this article, we have reviewed the advances and concerns, particularly the selectivity of AMPs for specific detection of pathogenic bacteria. This review also focuses the state-of-the-art mechanisms, challenges and prospects for designing potential AMP conjugated biosensors. The application of AMP in different biosensing transducers such as electrochemical, optical and piezoelectric varieties has been widely discussed. We argue that this review would provide insights to design and construct AMP conjugated biosensors for the pathogenic bacteria detection.

1. Introduction

The detection of pathogenic bacteria using a conventional approach such as microscopic visualization, culture-based identification, biochemical tests or molecular analysis requires either lengthy time, specialized laboratories, or expensive equipment (Burlage and Tillmann, 2017; Hameed et al., 2018). Although the microscopic determination of bacteria is relatively quick and cheap, the identification of pathogenic bacteria could not be resolved as it does not offer selective detection of bacteria. Moreover, the microbial staining for microscopic visualization of bacteria requires a proper bacterial smear that can be difficult to learn. Improper bacterial smear could result in over-staining, break cell wall or destroy cells, or even loss of morphological characteristics of the bacterial cell (Jayan et al., 2019). Furthermore, the culture-based detection of pathogens varies in their detection limits and is a time-consuming process for the bacterial cell to develop a visually observable cell density on selective media (colony-forming units on agar media or turbid liquid media) that may require even several days (Roda et al., 2012). Nonetheless, not all bacteria can be grown in laboratory conditions using conventional culture media (Ahmed et al., 2014; Hameed et al., 2018). Immunological tests and biochemical assays such as enzyme-linked immunosorbent assay (ELISA) and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) are attractive methods for detecting specific bacteria (De Bruyne et al., 2011; Hameed

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Table 1
An overview of conventional (culture-dependent/independent) and biosensor-based detection of pathogenic bacteria.

Conventional bacteria detection							
Detection techniques	Advantages	Limitations	Representative bacteria detected	Sample pre-concentration time	Detection time	Limit of detection	References
Lateral flow immunoassay	-Reliable -Cost-effective -Easy to handle -Highly specific and sensitive -Allows bacterial toxin detection	-Antigens or antibodies labelling is a pre-requisite of the technique	*Salmonella typhimurium* *Salmonella enterica* serovar *typhi*	12 h 6 h	– 10 h	4.6×10^7 CFU/mL 10^4–10^5 CFU/mL	(Shukla et al., 2014) (Kumar et al., 2008)
Conventional PCR	-Highly specific -Highly sensitive -Can be automated operations	-Requires purified DNA -PCR inhibitors interrupt the detection -Unable to distinguish live and dead bacteria	*E. coli* and *Shigella* spp.	8 h		1–2 Cells/ 100 mL	(Tsen et al., 1998)
Multiplex PCR	-Allows faster detection -Capable of multiple bacteria detection -Not required sample culture -Sensitive, specific and accurate	-The design of the primer is complicated -Highly accurate thermal cycler is required -Trained personnel in the laboratory is required for the detection -'primer biases' make it inappropriate for comparative measurements	*Salmonella enteritidis, Salmonella* spp. *Staphylococcus aureus, E. coli O157, Salmonella* spp. *Listeria monocytogenes, Yersinia enterocolitica*	24 h 24–48 h	– 15 min	10^5 CFU/ mL 10^1–10^3 CFU/mL	(Shi et al., 2010) (Guan et al., 2013)
Real-time PCR	-Rapid detection technique -Real-time bacteria detection and quantification -Highly sensitive, specific with attractive reproducibility	-Expensive equipment/ reagents are required along with highly-trained personnel's	*L. monocytogenes, Salmonella* spp. *S. aureus, Salmonella, Shigella* *Conjugated E. coli* DH5α	24 h – Overnight	1 h – <3 h	5 CFU/25 g 9.6 CFU/g 2.0 CFU/g 6.8 CFU/g Low	(Shi et al., 2010) (Ma et al., 2014) (Nayak et al., 2013)
NABSA	-Highly sensitive and specific -Lower cost -Capable of distinguishing live and dead bacteria	-Viable bacteria are required -RNA handling is complicated	*S. typhimurium* *S. enteritidis*	24 h	<90 min	10 CFU/ mL	(Mollasalehi and Yazdanparast, 2013)
LAMP	-A large number of DNA copies could be produced in less than one hour -Easy to operate -Highly sensitive -High specificity -Low cost -Sophisticated equipment is not required -Thermal cycling system is not required	-Only a few microorganisms can be detected -Primer design is sophisticated -Could not detect un-sequenced or unknown targets	*Vibrio vulnificus* *Vibrio parahaemolyticus* *V. parahaemolyticus*	6 h Overnight 12 h	8 h 1 h 1 h	5.4 CFU per reaction 10 CFU/ reaction 7.2 copies/μL	(Han et al., 2011; Singhal et al., 2015) (Wang et al., 2013) (Xia et al., 2016)
MALDI-TOF	-Rapid detection with high accuracy -Less expensive compared to molecular-based detections -Trained personnel and sophisticated laboratory is not emergent	-High installment of MALDI-TOF equipment	*Leuconostoc* spp., *Fructobacillus* spp., *Lactococcus* spp.	24 h	<1 h	–	(De Bruyne et al., 2011)
DGGE	-Can compare microbial diversity	-Fewer number of microbes leads to the low resolution	*Bacillus pumilus, Bacillus megaterium, Bacillus thuringiensis, B. firmus*	48 h	–	10 CFU/ mL	(Garbeva et al., 2003)

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Table 1 (*continued*)

Conventional bacteria detection								
Detection techniques		Advantages	Limitations	Representative bacteria detected	Sample pre-concentration time	Detection time	Limit of detection	References
Metagenomics approach		-Can identify a few unknown bacterial species -Can randomly detect bacteria -Can quantify bacteria	-Data processing and interpretation are complicated along with diverse -Acquisition and analysis of data are time-consuming -Highly trained personnel and laboratory is required	*Borrelia* spp., *Rickettsia* spp., *Candidatus* spp., *Neoehrlichia* spp.	-	<48 h	–	(Carpi et al., 2011)
ELISA		-Highly specific -Rapid and time saving due to automated operation -Allows bacterial toxin detection -Large numbers of samples can be measured	-Lower sensitivity -False-positive or negative results -Possibility of cross-reaction with related epitopes -Enrichment is a pre-requisite -Antigens or antibodies labeling is required	*E. coli* O157	24 h	3 h	68 CFU/mL in PBS and 6.8×10^3 CFU/mL in food samples	(Shen et al., 2014)
DNA microarray (Coupled with qPCR)		-Highly specific and sensitive -Could detect multiple pathogens	-Associated with high cost -Cannot distinguish between live and dead bacteria -Non-specific hybridization reduce the detection efficiency	*E. coli*	–	–	5 ng of *E. coli* DNA	(Deshmukh et al., 2016)
FISH		-Allows high selective detection -Could distinguish live and dead bacteria -Allows rapid and direct detection -Capable to detect bacteria with high specificity compared to conventional staining techniques	-Lower sensitivity -Pre-enrichment is required -May give false-negative/positive results -Associated with high cost -Detection is associated with the availability of the specific antigen	*P.aeruginosa*, *S. aureus*, *Streptococcus* spp., *Micrococcus* spp.	24–28 h	–	–	(Malic et al., 2009)
Biosensors based detection								
Optical biosensors	SPR/SERS	-Highly sensitive -Permits real-time or close to real-time detection -Label-free detection	-Associated with high cost -Sensitive to the surrounding environment (*i. e.* pH, buffer, temperature) -Surface modification is challenging -Requires the use of a heavy device	*Legionella pneumophila*	–	30 min	1×10^3 CFU/mL	(Manera et al., 2013)
				E. coli O157	9–12 h	16 min	5 CFU/25 g of food samples	(Mondani et al., 2016)
				S. enterica serovar Typhimurium	24 h	N. A	15 CFU/mL	(Duan et al., 2016)
				E. coli O157	Overnight	1 h	1×10^2 CFU/mL	(Najafi et al., 2014)
	Colorimetric	-Detection transducers are not required - Low cost	- Not quantitive - Low sensitivity	*V. parahaemolyticus*	18 h	45 min	2.4 CFU/mL	(Sadsri et al., 2020)
Electrochemical biosensors	Amperometric	-Simple -Possible to miniaturize -Low cost	-Need redox elements to enhance curret production -Sensitive to the surrounding environment (i.e., pH, buffer)	*L. pneumophila*	5 days-	3 h	10^4 CFU/mL	(Martín et al., 2015)
	Potentiometric	-Can handle multiple samples	-Sensitive to the surrounding	*S. aureus*	48 h	N. A	8×10^2 CFU/mL	(Zelada-Guillén et al., 2012)

(*continued on next page*)

Table 1 (*continued*)

Conventional bacteria detection								
Detection techniques		Advantages	Limitations	Representative bacteria detected	Sample pre-concentration time	Detection time	Limit of detection	References
		- Real-time detetion	environment (i.e., pH, buffer)					
	Impedimetric	-Simple -Real-time detection	-It is not an automated technique -Require bulky devices -Sensitive to the surrounding environment (i.e., pH, buffer) -Require theoretical stimulation for data analysis	*L. monocytogenes*	19 h	3 h	10^4–10^5 CFU/mL	(Kanayeva et al., 2012)
Mass-based biosensors	Cantilever	-Ability to detect one more analyte -Easy to handle -Permits real-time detection	- Lower sensitivity -Requires multiple washing & drying steps	*S. typhimurium*	–	N. A	1×10^3 CFU/mL	(Shi et al., 2017)
	QCM	- Real-time -Simple -High compatibility with point of care devices	-Lower reproducibility due to problematic crystal surface regeneration -Time-consuming due to long incubation time with bacteria -Sensitive to the surrounding environment (i.e., pH, buffer)	*Bacillus anthracis*	–	N. A	1×10^3 CFU/mL	(Hao et al., 2009)
Hybrid biosensors (Optical + Electrochemical)		-Require short times -Easy to handle -Possibility of regeneration -Label-free detection	-Low specificity -Moderate sensitivity	*L. pneumophila*	72 h	45 min	2.3×10^2 CFU/mL	(Islam et al., 2020)

DGGE: denaturing gradient gel electrophoresis, PCR: polymerase chain reaction; qPCR: quantitive polymerase chain reaction; MALDI-TOF; matrix-assisted laser desorption ionization time-of-flight,NABSA: nucleic-acid sequence-based amplification; LAMP: loop-mediated isothermal amplification; FISH: fluorescence in situ hybridization, SPR: Surface plasmon resonanance; SERS: Surface enhanced Raman spectroscopy.

et al., 2018). However, they are costly and time-consuming, requiring advanced technical skills and expertise for data interpretation (Burlage and Tillmann, 2017). The molecular analysis allows the detection of specific bacteria using the genetic material of bacteria; however, it is an expensive and sophisticated procedure (Jayan et al., 2019). Although the real-time polymerase chain reaction (PCR) technique requires short analytical time (a few hours), it is associated with trained users along with specialized equipment (Oblath et al., 2013). In addition, sample enrichment and DNA purification are needed for reverse transcriptase-polymerase chain reaction (RT-PCR) (Burlage and Tillmann, 2017; Oblath et al., 2013). Hence, it is emergent to develop a fast, sensitive, easy to handle and cost-effective method, to detect pathogenic bacteria. The detailed advantages and limitations of several conventional biosensor-based bacteria detection are described in Table 1.

Rapid detection of pathogenic bacteria has renewed attention due to the significant impact on public health (Pinel et al., 2021; Reuter et al., 2020). However, major rapid detection techniques contain different procedures such as pre-enrichment, signal transmission, and data analysis (Zhang et al., 2018). The pre-enrichment is considered to be a time consuming modules, which aims to pre-concentrate bacteria in less quantitaty of solution (Zhang et al., 2018). Total detection or processing time includes each individual step, from pre-enrichment to final results. A majority of the rapid detection systems widely practice pre-enrichment process (Kumar et al., 2008; Mursaloğlu and Çevik, 2021; Myint et al., 2006) for the detection of bacterium; however, the detection time is reported by solely considering a read-out of the final results. For instance, the final detection of bacteria through MALDI-TOF analysis could be completed in less than an hour; however, pre-enrichment procedure is usually required before the final detection (Mursaloğlu and Çevik, 2021) (Table 1). Similarly, most of the molecular-based and immunological methods such as ELISA (Kumar et al., 2008), PCR (Myint et al., 2006), fluorescence in situ hybridization (FISH) (Vieira-Pinto et al., 2008), lateral flow immunoassay (LFA) (Singh et al., 2015) require sample pre-enrichment step before final results read-out (Table 1). Therefore, a rapid bacterial detection method with minimal pre-enrichment is highly desirable to reduce the detection time and its practical applicability.

Biosensor-based detection of pathogenic bacteria can be advantageous compared to conventional methods (Hoyos-Nogués et al., 2018; Roda et al., 2012) as it requires minimal pre-enrichment of sample. Besides, biosensor allows rapid, robust, cost-effective, and portable detection than other methods (Lv et al., 2018; Wisuthiphaet et al., 2019). However, bio-recognition plays a crucial role to obtain an accurate signal from biosensors (Elakkiya and Matheswaran, 2013; Ramanavičius et al., 2006). Generally, several bio-recognition elements such as antibodies (Ab), aptamers, carbohydrates, peptides, and/or a mixture of these have been used in biosensor design (Hoyos-Nogués et al., 2018). Among these, antibodies have been considered an attractive option

because of their highly specific interaction with antigenic target sites (Etayash et al., 2013; Mannoor et al., 2010). Recent studies have shown that Ab-based biosensors can successfully detect low concentrations of pathogenic bacteria (Aziziyan et al., 2016; Li et al., 2012). However, it has been known that antibodies suffer from a lack of stability, especially during the detection of pathogens under harsh environment (Leonard et al., 2003; Templier et al., 2016). Additional binding agents such as biotin and neutravidin can enhance surface coverage with Abs, which would increase the probability of trapping the bacterial antigens efficiently with multiple sites (Aziziyan et al., 2016). However, the addition of an extra interface to the binding architecture in the vicinity of the sensor substrate might reduce the reproducibility of sensor performance. In addition, the distance between the biochip surface and captured bacteria in the Ab-based bio-architectures is at least at 15–18 nm, which could result in a less sensitive response of biosensors designed for electrostatic detection of bacteria and/or sensing the charge transfer to/from bacteria (Islam et al., 2020, 2021).

Recently, antimicrobial peptides (AMPs) have been reported as promising bio-recognition elements in the biosensing platforms (de Miranda et al., 2017; Dong and Zhao, 2015; Etayash et al., 2013). AMPs are small peptide fragments that are expressed in both prokaryotes and eukaryotes (Pinto et al., 2019; Qiao et al., 2020a). AMPs contain multiple niches that enable them to serve as recognition elements by binding with high affinity to bacteria and fungi (Mannoor et al., 2010). The increased stability of AMPs compared to typical globular proteins is also considered an attractive option for biosensing applications (Etayash et al., 2014b; Mannoor et al., 2010). It has also been reported that some cationic AMPs show efficient binding ability even after extreme treatments, such as autoclaving and boiling (Etayash et al., 2014a; Mannoor et al., 2010). Some AMPs, such as Magainin I (Kulagina et al., 2005), Clavanin A (Andrade et al., 2015), and polymyxin B (Kulagina et al., 2006), have been utilized as bio-recognition probes. In related studies, AMPs have shown a semi-selective binding nature to the target cells. Hence the sensors suffered from a lack of specificity (Mannoor et al., 2010). In contrast, some other studies of AMPs have shown high specificity to the target cells (Hossein-Nejad-Ariani et al., 2018a). Mannoor et al. (2010) reported that the gold electrode functionalized with Magainin I showed differential binding affinity to pathogenic *Escherichia coli* (*E. coli*) O157, *Listeria monocytogenesis* (*L. monocytogenesis*) and *Salmonella typhimurium* (*S. typhimurium*). Hossein-Nejad-Ariani et al. (2018b) reported that a gold microelectrode functionalized with Leucocin A (Leu A) showed high binding affinity to L. *monocytogenes*. High binding affinity of AMPs towards the target bacteria allows the detection of bacteria at a low concentration through the AMP-anchored biosensors (Mannoor et al., 2012; Mannoor et al., 2010).

Despite AMPs have been widely used in the past few years as bioreceptors for capturing bacteria, the mechanism of interaction between AMP and bacteria is not eminent. Initially, the positively charged peptide may interact with the negatively charged bacterial membrane via the electrostatic interactions; consequently, leading to the interaction of the peptide with a specific membrane moiety or receptor located on the bacterial surface (Onaizi and Leong, 2011). However, the binding affinity between peptides and bacteria depends on multiple factors (Etayash et al., 2014a; Etayash et al., 2014b). For instance, the mannose-phosphotransferase system (man-PTS) is considered as one of the predominant factors since the expression level of man-PTS receptors can vary from species to species, which would confer specificity (Etayash et al., 2014b). Besides receptors, several recent studies have reported that the inherent property of the bacterial membranes may regulate specific peptide-bacterial interactions (Verdon et al., 2008). Even further, it had been speculated that the number of receptors and their compositions present in the bacterial cell membrane play crucial roles for a specific interaction with AMPs (Marchand et al., 2011). It is interesting to note that the immobilized peptides may interact more efficiently with the bacterial membrane compared to the peptides in solution (Hilpert et al., 2009). This is, however, difficult to conclude from the limited number of studies that have been conducted so far (Bagheri et al., 2009; Cho et al., 2007; Costa et al., 2011; Haynie et al., 1995).

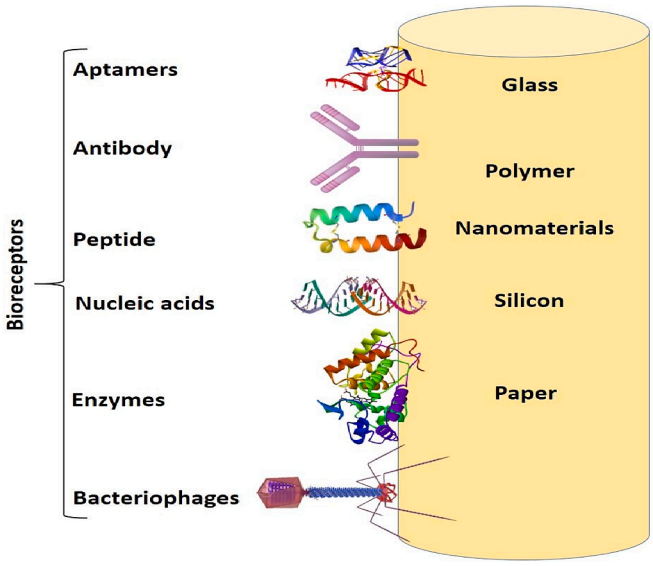

Fig. 1. Graphical representation of different types of bio-recognition elements on different surfaces for bacteria detection.

AMPs have been proposed as potential short ligands in biosensing technique due to their stability and bacteria capturing efficiency (Mannoor et al., 2010; Qiao et al., 2020b). However, the specificity of AMP is considered as a limiting factor and very limited studies addressed this issue. Therefore, in this paper, we have reviewed recent advances and concerns of biosensors, particulary the selectivity of AMP-anchored biosensors, for detecting pathogenic bacteria. Furthermore, this review presents the state-of-the-art mechanisms, challenges and prospects for designing AMP conjugated biosensors. The application of AMP as ligand in different biosensing transducers such as electro-chemical, optical, and piezo-electric for the detection of pathogenic bacteria has been widely discussed in this paper. Of note, some promising aspects such as automated, in-situ, and rapid detection of pathogenic bacteria through the AMP-based biosensors are also highlighted in the paper.

2. Bio-receptors used in detecting pathogenic bacteria

Bioreceptors play a crucial role in biosensing, as they recognize targeted analytes and also determine the efficiency of sensors through the specificity and sensitivity (Justino et al., 2015). Therefore, the performance of a sensor is determined by the selection of bioreceptors (Hoyos-Nogués et al., 2018). However, the stability of bioreceptors strongly influences the binding affinity and performance of biosensors (Templier et al., 2016). Bioreceptors ranging from small molecules (i.e., enzymes, short peptides, sugars, and nucleic acid) to large molecules (i.e., proteins, viruses, and whole cells) are widely being used for detecting bacteria (Hoyos-Nogués et al., 2018; Qiao et al., 2020a). Several representative biomolecules used as bioreceptors are presented in Fig. 1.

In general, large molecules, especially proteins and bacteriophages, have shown higher specificity compared to the smaller molecules (Templier et al., 2016). In contrast, synthetic and small molecules are found with higher stability and could be easily optimized (Cambray et al., 2018). However, none of them are considered a perfect candidate to make the sensors practically applicable. The advantages and limitations of different bio-recognition elements used in biosensing platforms are discussed in Table 2.

In biosensing technology, antibodies are widely used for bacteria detection due to high specificity in pathogen seizing as well as recognition (Byrne et al., 2009). The polyclonal, monoclonal, and

Table 2
An overview of different bio-recognition elements used for bacteria detection.

Category	Description	Advantages	Limitations	Reference
Antibody	A specialized protein mainly produced by plasma cells	• Allows to detect different kinds of cells and cell metabolites • Allows highly specific reaction between antigen-antibody conjugates • Allows direct recognition • Allows non-invasive interaction	• They are not capable of distinguishing between live and dead bacterial cells • Batch-to-batch consistency is found, especially in the case of polyclonal antibody • Longer times and higher costs are required to produce them • Cannot recognize after slight modification in the target analytes • Lower stability in low or high pH, ionic solutions, and high temperature	(Bazin et al., 2017; Liu et al., 2018)
Enzymes	A specialized protein that binds to the allosteric site	• Detecting target analyte based on catalytic activity • They are highly stable even for a year • Strongly bind with the target analytes • Stable in extreme environments such as high temperature and high or low pH	• Require more than one assay steps • Show less stability under extreme environmental conditions (e.g., temperature, pH) • In some cases, interfere with endogenously produced enzymes by target analytes • Catalytic efficiency predominantly depends on the sequence of the DNA	(Habimana et al., 2018; Hwang et al., 2016)
Nucleic acids	Small biomolecules are known as DNA and RNA	• Determine target analytes based on complementary nucleic acid sequences, hence it allows highly specific detection • Nucleic acids could be promptly extracted and regenerated compared to typical antibody, enzymes, and proteins production	• Costly methods • They are less soluble in the water environment • Not stable in harsh environment (sensitive to pH and temperature) and difficult to make portable and automated sensors using them	(Habimana et al., 2018)
Aptamer	Short and single-stranded highly specific and sensitive synthetic oligonucleotides	• Recognition of analytes based on shape but not sequences • They are highly stable and easy to modify • Enhances interaction due to possession of multiple functional groups • Rigid backbone • Capacity to confer a cellular phenotype	• False-positive results are seen while dealing with large molecules • Semi-selective binding	(Habimana et al., 2018; Mascini et al., 2012).
Antimicrobial peptides	A small peptide sequence	• Highly stable and could be produced in a large quantity using synthetic methods • Multiple niches are advantageous to capture a significant number of target analytes	• In most of the cases, they are semi-selective or non-selective • They are not considered as efficient for a long time since they are invasive to the target analytes	(Habimana et al., 2018)
Bacteriophages	A special virus is generated via infecting host bacteria	• Wide-range libraries • Allows to genetically modify after selection • Allows selection against a toxic or low immunogenic target	• Unable to recognize gram-negative bacteria because of thick outer membrane • Targets are required to be immobilized for identifying the efficiency of phages • The immobilization of bacteriophage is considered challenging among biosensors substrate due to weak interactions.	(Harada et al., 2018; Schmelcher and Loessner, 2016)
Molecular imprinted polymers	Synthetic polymer-based receptors	• Detain original properties in an extreme thermal and chemical condition • Not easily degraded by enzyme • Can be handled both aqueous and organic solvents	• Highly skilled personnel are required to produce them • The production process is time-consuming	(Dinc et al., 2019)

Table 3
Comparison of different types of antibodies.

	Polyclonal	Monoclonal	Recombinant
Production time	2–3 months	4–6 months	1 week
Reproducibility	Limited	High (in the same batch)	Very high
Specificity	Specific	Highly specific	Very high specificity
Binding mechanism	Multiple	Single	Single
Stability	Moderate	High	Moderate
Cost	Moderate	High	Very high

recombinant antibodies could be distinguished based on their selective natures and their production methods as shown in Table 3. Among the three types of antibodies, monoclonal antibodies are found to be more advantageous compared to others due to their high specificity. The antibody is preferred as a bio-receptor because it is simple and easy to handle during bio- functionalization of sensor substrates.

Furthermore, antibodies allow not only to detect entire bacterial cells but also cell by-products and metabolites (Byrne et al., 2009). However, the differentiation between live and dead cells were not achieved through the antibody based techniques (Etayash et al., 2014b). In addition, several limitations such as batch-to-batch inconsistency, production challenges, and high cost, restricted their application in biosensing technology (Etayash et al., 2013).

Nucleic acids (NA) allow quick and specific detection of pathogenic bacteria with a lower detection limit, as low as a single bacterium. The versatility of NA-based methods allows for designing a specific probe sequence typically in the length of 10–20 basepairs to target a selective gene of bacteria (Lui et al., 2009). In NA-based bio-recognition elements, DNA is considered as an attractive signal transducer as it contains a negative charge, therefore, producing promising results through the electrical measurements over other typical biosensing detections (i.e., optical and mechanical measurements) (Riedel and Lisdat, 2017). NA-based detection techniques have shown higher sensitivity compared to others; hence, high-level quality control is required to prevent contamination (Bal et al., 2017). In addition, effective sample preparation is an essential prerequisite for the successful target analyte detections. Besides, inhibitors present in the sample, and the deteriorated

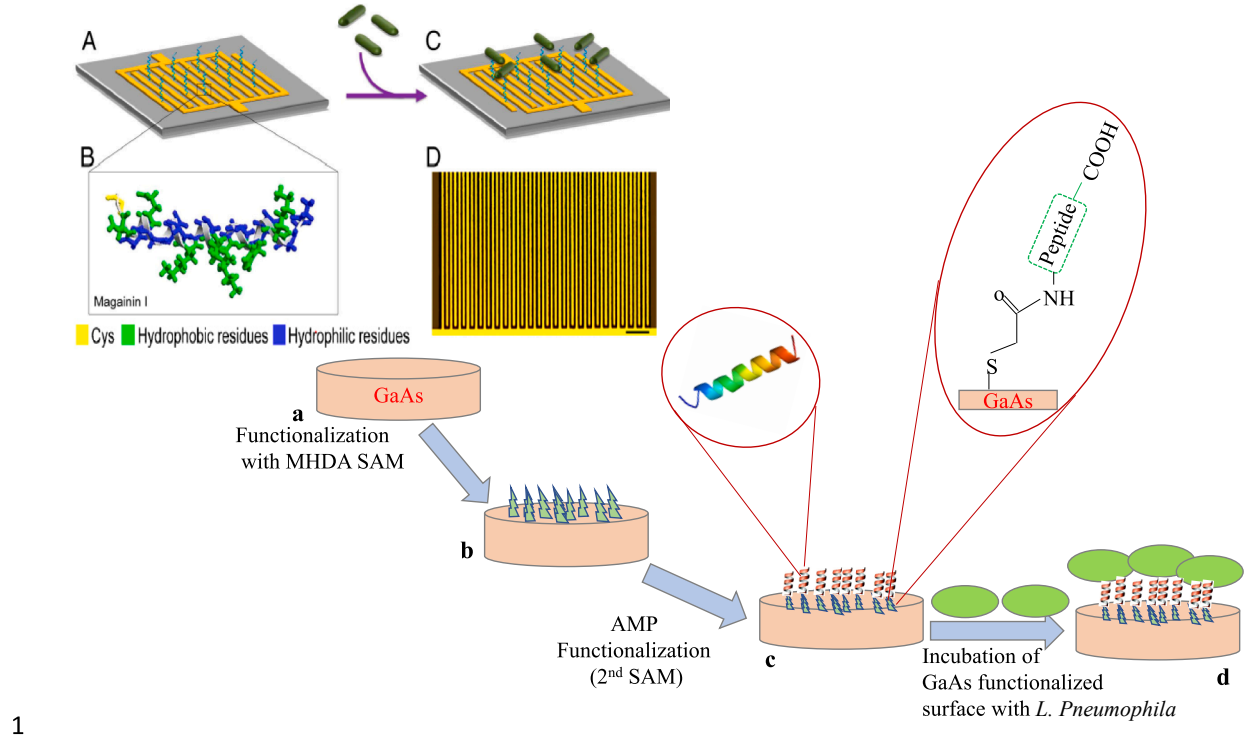

Fig. 2. A schematic of representation of immobilized of AMPs on the surface micro-electrode array biosensors (A-D), the helical structure of AMP is presented as a magnified image (2B), the terminal AA is modified with cysteine residue which determines the hydrophilic and hydrophobic faces of AMP); the bio-recognition mechanism of AMP (a-d), a) non functionalized GaAs wafer, b) functionalized with MHDA SAM, c) AMP functionalized following the EDC/NHS step, c') globular structure of AMP, c") MHDA-peptide hybrid, d) attachment of bacteria to peptide, d') interaction between peptide and bacterial cell membrane (Islam et al., 2020; Mannoor et al., 2010).

DNA could lead to false-negative/positive results (Maffert et al., 2017).

The aptamers are short, single-stranded, highly specific and sensitive synthetic oligonucleotides, which can specifically bind to target analytes (e.g., toxins, bacteria, proteins, and hormones) (Kaur et al., 2018). Generally, aptamers are synthesized via systemic evolution of ligands by exponential enrichment (SELEX) technique; an efficient screening approach, where the desired aptamers could be successively determined from the oligo-nucleic acid library using repeated cycles of amplification (Habimana et al., 2018). In the case of aptamer synthesis, nucleic acids (i.e., DNA or RNA) intended to bind with targeted analytes must be selected from a library that is sequenced and amplified to obtain specific aptamers (Lim et al., 2005). Aptamer functionalized biosensors are named as aptasensors detecting pathogenic bacteria with several advantages particularly, ease of modification along with promising stability (Majdinasab et al., 2018). However, aptasensors are not preferred to detect large molecules such as an whole bacteria as they might provide false-positive signals (Saha et al., 2012).

Bacteriophages are considered as efficient bio-recognition elements as they allow fast and highly selective detection of bacteria (Richter et al., 2018; Singh et al., 2012). In the biosensing technique, the natural affinity of bacteriophages towards the host bacterial cells can be utilized to design highly specific biosensor substrate (Richter et al., 2018). In addition, the bacteriophages can survive and retain their activity in high temperatures (up to 76 °C) and inorganic solvents (Richter et al., 2018). Hence, they are considered as robust bioreceptors compared to others (Brigati and Petrenko, 2005). Additionally, unlike antibodies, a larger quantity of phages could be easily and inexpensively produced. A significant number of phages can be generated by simply infecting a bacterium with a phage. Thus, the phages are considered as promising bio-recognition elements in biosensor-based detection of pathogens (Richter et al., 2018). Besides, the phage-based biosensors can distinguish live and dead cells as the phages can replicate solely within the viable bacteria. However, several limitations are evolved with the uses of phages in biosensing technology, especially difficulty to immobilize on the sensor surface and also the lower stability (Aliakbar Ahovan et al., 2020). The effective immobilization of phage-derived recognition elements or phage to the sensor surface is crucial to design an efficient phage-based biosensor. Several techniques such as covalent, oriented immobilization, and physical absorption have been widely practiced for phage immobilization (Schmelcher and Loessner, 2014; Singh et al., 2012). However, in most cases, adsorption is hampered due to the inconsistent density and weak binding of recognition ligands attached to the sensor substrates (Schmelcher and Loessner, 2014).

Molecularly imprinted polymers (MIPs) are another known bio-receptor which is a kind of matrix of polymer and avail target specificity of analyte in several ways such as size exclusion or inclusion, electrostatic interactions non-covalent bonding (Cieplak and Kutner, 2016). The turbidity of MIPs regulated by several factors such as functional monomers, target bio-analytes, crosslinkers, and solvents (Wackerlig and Schirhagl, 2016). Generally, MIPs are designed to capture target analytes, as they can form synthetic bio-recognition between the polymer matrix and bio-analyte. Unlike typical bio-recognition elements, MIPs are synthetically fabricated for an individual target bio-analyte (Hoyos-Nogués et al., 2018). In other words, the in situ synthetic polymer-based bio-recognition elements could be designed to develop a biosensor (Sharma et al., 2015). Hence, the main advantage of MIPs is the specific interaction between bio-analyte and bio-recognition element, which is not necessarily to be biochemically identified. However, the stability of polymer is a major concern in designing MIP-based biosensors for bacterial pathogen detection.

3. The use of AMP as a ligand of biosensors

AMPs generally exist in the host immune system to eliminate wide ranges of pathogens such as viruses, bacteria, fungi, and even cancer cells (Ageitos et al., 2017; Nguyen et al., 2011). Most of the cationic

Table 4
AMPs used as bio-recognition elements for bacterial detection.

Technique	Targeted bacteria	Substrate immobilization	AMPs	Analysis time (min)	Sample type	Detection limit (CFU/mL)	Selectivity of Gram positive/ Gram negative	Exposed bacteria	Specificity	References
Electrochemical method	*E. coli O157*	Gold surface – lipoic acid N-hydroxysuccinimide – ferrocene	Magainin I	90	PBS	10^3	Gram negative	*E. coli* O157, *E. coli* K12, *Staphylococcus pidermidis* and *Bacillus subtilis*	Showed higher selectivity against *E. coli* O157	(Li et al., 2014)
	E. coli O157 *S. typhimurium*	Gold surface – cysteine	Magainin I	15	PBS	10^3	Gram negative	*E. coli* O157, *S. Typhimurium*, nonpathogenic *E. coli*, *L. monocytogenes*	Showed higher selectivity against *E. coli* O157	(Mannoor et al., 2010)
	L. monocytogenes	Gold surface – cysteamine	Leucocin A	20	10% milk	10^3	Gram positive	*L. monocyotogene*, *S. aureus*, *L. innocua*, *E. faecalis*	Selectively detected *L. monocytogenes* from other Gram-positive strains	(Etayash et al., 2014a)
	E. coli	-	Colicin V	15	water	10^3	Gram negative	*L. monocyotogene*, *S. aureus*, *L. innocua*, *S. enteritidis*, *P. fluorescens*	Showed higher selectivity against *E. coli*	(K. Jiang et al., 2015)
	E. coli , *K. pneumonia E. faecalis*, *B. subtilis*	-	ClavA	N/A	PBS	10^2	Gram negative	*E. coli*, *K. pneumonia*, *E. faecalis*,*B. subtilis*	Showed higher specificity against *E. coli*	(Andrade et al., 2015)
	L. monocytogenes	-	Leucocin A	60	seawater	10	Gram positive	*L. monocytogenes*, *L. iuanuii*, *S. typhimurium*, *E. coli* O157	Selectively detected *L. monocytogenes*	(Lv et al., 2018)
	P. aeruginosa	Gold surface – cysteine	G10KHc			10^5	Gram negative	*P. aeruginosa*, *Streptococcus mutans*	Selectively reacted with *P. aeruginosa*	(Lillehoj et al., 2014)
	Streptococcus mutans	Gold surface – cysteine	C16G2cys			10^5	Gram positive	*P. aeruginosa*, *Streptococcus mutans*	Selectively reacted with *Streptococcus mutans*	(Lillehoj et al., 2014)
Fluorescent method	*E. coli O157*	-	polymyxins	90	PBSTB	5.0×10^4	-	*E. coli* and *Salmonella*	Not reported	(Kulagina et al., 2005)
	E. coli O157	-	Cecropin P1	30	PBS	10^3	-	-	Not reported	(Arcidiacono et al., 2008)
	Non-pathogenic *E. coli*	Glass microbeads – N-[γ-maleimidobutyryloxy] succinimide ester (GMBS) – cysteine	Magainin I			10^3	-	-	Not reported	(J. H. Yoo et al., 2014)
olorimetric method	*E. coli O157*	-	Magainin I	45	spiked apple juice and ground beef	119, 451	Gram negative	*E. coli O157*, *V. parahaemolyticus*, *L. monocytogenes*, *S. aureus*, *S. Typhimurium*, *E. coli* DH5α, *E. coli* BL 21	Showed high specificity against *E. coli O157*	(Qiao et al., 2017a)
	E. coli O157	Magnetic nanoparticles	Magainin I	30	spiked apple juice and ground beef	84, 233	Gram negative	*E. coli O157*, *L. monocytogenes*, *S. Typhimurium*, *E. coli* DH5α, *E. coli* BL 21	Showed high specificity against *E. coli* O157	(Qiao et al., 2017b)
	E. coli O157, O26 and O111	-	Cecropin P1	15	spiked ground beef	10^5, 10^6 and 10^5	Gram negative	-	Reacted to *E. coli* O157, O26, and O111; not reacted to 19 non-*E. coli* strains	(Yonekita et al., 2013)
SPR	*E. coli O157*	Silver nanoparticles	Magainin I	40	water, fruit and vegetable juice	5.0×10^2	-	*E. coli O157*	-	(Zhou et al., 2018)
Hybrid Sensors (Optical + Electrochemical)	*L. pneumophila,*	Gallium arsenide	Warnericin RK	45	PBS	10^3	Gram negative	*L. pneumophila*	Showed high specificity against *L. pneumophila*	(M. A. Islam et al., 2020)
	E. coli O157	Gold	Magainin I	15 s	PBS	2.3 x 10^2	Gram negative	-		(Z. Li et al., 2015)

AMPs that are commonly interact with cell membranes of bacteria via electrostatic interactions, and subsequently, follow specific interaction with bacterial cell envelops (Brogden, 2005). Recently, AMPs have been applied as therapeutic agents, especially for killing bacteria, as they are capable to overcome bacterial resistance (Mahlapuu et al., 2016). Apart from their biological activity, AMPs are observed as highly stable molecules that could be produced in large quantities using several synthetic methods (Zasloff, 2002). It is interesting to note that these molecules have also been used as a bio-recognition element for selectively capturing target analystes particularly bacteria (Hoyos-Nogués et al., 2018). Generally, AMPs interact electrostatically with the negatively charged molecules and lipopolysaccharides (LPS) present on bacterial membranes (Brogden, 2005). The schematic diagram of interaction between peptides and bacteria is illustrated in Fig. 2. The interaction between AMPs and phosphate group of bacteria LPS has been exploited in biosensing technology addressing detection, quantification, and classification of bacteria (Fig. 2d') (Pavan and Berti, 2012). From the engineering perspective, AMPs demonstrate attractive properties that could be advantageous in designing biosensing devices. The AMP can be employed as spacers or chemo-selective anchors to selectively functionalize a diverse range of surfaces. These chemo-selective anchoring groups allow them to efficiently immobilize target analytes on the surface of sensor substrates. However, achieving sensitivity in complex real environments, particularly selectivity within mixed bacteria, is still challenging and requires extensive investigations.

Several recent studies have shown that AMPs could be used as bio-receptors for selectively detecting bacteria using biosensing techniques as presented in Table 4. For instance, Kulagina et al. (2005) detected two most harmful pathogenic bacteria, namely *S. typhimurium* and *E. coli* O157, using a fluorescence biosensor anchored with AMP Magainin I. In their subsequent study (Kulagina et al., 2006), they functionalized silanized glass surfaces using different AMPs such as cecropin A, magainin I, parasin, polymyxin B, and polymyxin E, and observed differential cell capture efficiencies for *S. typhimurium* and *E. coli* O157. They also observed that the non-pathogenic *E. coli* did not interact with most peptides. Their results clearly show that the AMP could be be used as bio-recognition elements for selective detection of bacteria even at species level. Furthermore, they investigated the efficiency of AMPs for detecting several bacteria such as *Brucella melitensis* and *Coxiella burnetii*, where they observed maximum binding affinity of *B. melitensis* by the bactenecin, polymyxin B and polymyxin E functionalized surface while no binding affinity was observed for magainin I functionalized surface (Kulagina et al., 2007). Therefore, this study further strengthens the selectivity of AMPs to distinguish pathogens in closely related species.

The lower limit of detection (LOD) has been reported in several AMP-based biosensing studies as depicted in Table 4. Few of studies have shown that very low LOD can be obtained by AMP-conjugated biosensors. For instance, Lv et al. (2018) detected 10 CFU/mL of L. *monocytogenes* using an AMP pair based sandwich technique. In an another study, Albanese et al. (2019) detected 1.5 CFU/mL of *Listeria innocua* using an AMP (nisin) functionalized electrochemical sensors. It has also been reported that the AMP-based colorimetric biosensor can detect 13 CFU/mL of *E. coli* O157 (Qiao et al., 2017b). These results are promising to apply the AMP-based biosensors in the practical field. However, in most cases, the LODs are reported by solely considering dilution factors rather comparing with positive or negative control samples such as other bacterial strains. Besides, in some studies (Li et al., 2015; Mannoor et al., 2010; Qiao et al., 2017a), the detection time is reported based on final read-out of biosensing results. However, the detection time should be recognized either by considering whole process including sample preparation to final results or the time of individual stages to better assess the LODs in the context of realistic conditions, where the target bacteria would exist with other bacterial strains.

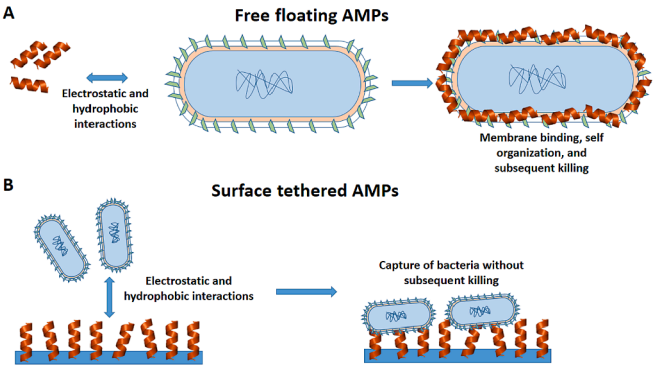

Fig. 3. Techniques for capturing bacteria on the surface of biosensors substrate, A) AMP-bacteria pre-mix technique, B) surface tethering technique (Pardoux et al., 2020).

4. Technique of bacteria-capture using AMPs

Generally, AMPs have been tethered on the surface of biosensors for capturing bacteria (Islam et al., 2020; Jiang et al., 2015). However, bacteria could also be captured on the surface of biosensors using the AMP-bacteria premix technique as presented in Fig. 3a (Pardoux et al., 2020). In this technique, AMPs are immobilized on the surface of bacteria via utilizing electrostatic and hydrophobic interactions between peptides and bacterial envelopes. The surface tethering technique has widely been used to capture bacteria on the surface of biosensors substrate as shown in Fig. 3b (Mannoor et al., 2010). In this technique, several physical and chemical (i.e., covalent interaction) methods have been used to immobilize AMPs on the surface of biosensing substrates. In physical methods, layer-by-layer assembly of AMPs on the solid interface is the most attractive approach (Shukla et al., 2010).

In this technique, the number of AMPs polymer layers could be designed flexibly, which allows for controlling peptide loading on the solid surface (Costa et al., 2018). However, the charge diffusion could be interrupted due to the polymer surfaces (Sanchez-Gomez and Martinez-de-Tejada, 2017). In addition, this technique also suffers due to the lower stability (Ferreira and Zumbuehl, 2009). Hence, this assembly needs to be carefully designed by considering the afore-mentioned problems. It is worth noting that the physical adsorption technique is widely used for tethering AMPs to create antimicrobial surfaces (Kugel et al., 2011). However, this is comparatively less used technique for functionalizing AMP on biosensors surfaces (Hoyos-Nogués et al., 2016). Alternatively, the covalent based immobilization of AMPs on biosensor surfaces is the most used technique to tether peptides on biosensor surface (as presented in Table 5) as it is more effective compared to physical immobilizations (Bagheri et al., 2009). In the covalent method, the AMPs could be functionalized with several spacers (i.e., thiol) containing reactive groups to immobilize the peptides on the biosensor surface as presented in Fig. 4. In covalent based coupling, one of the most effective approaches is to immobilize peptides on the biosensor surfaces through reactive groups present on the self- assembled monolayer (SAM).

The layer of SAM could be functionalized with several reactive coupling reagents (Sanchez-Gomez and Martinez-de-Tejada, 2017). The length of carbon chain present in SAM could be designed from one to several carbons, by considering which length is more effective for effectively reacting with AMPs (Stassen et al., 2017). The polymer resins, especially polyethylene glycol (PEG), containing reactive groups corresponding to several peptides is another common approach for covalently immobilized AMPs (Abshar Hasan et al., 2020). In general, PEG allows faster immobilization, which can substantially increase peptideand bacteria interactions leading to the enhancement of the performance of biosensors (Karimzadeh et al., 2018). However, in the PEG-based peptide bound, there is a possibility of polymer degradation

Table 5
An overview of the AMP tethering techniques on the biosensor's substrates.

Name	Time	Immobilization technique	Length	Structure	References
Magainin I	60 min	Cysteine terminated peptide was covalently (directly) immobilized on the gold electrode surface	23	α-Helix	(Mannoor et al., 2010)
Clavanin A	35 min	The peptide was covalently immobilized with EDC-NHS activated cysteine thiol	37	α-Helix	(de Miranda et al., 2017)
Plantaracin 17C	60 min	The peptide was covalently immobilized with EDC-NHS activated (3-mercaptoproyl) trimethoxy silane (MPTS) thiol	17	α-Helix	(Guralp et al., 2015)
GIGKFLHSAGKGKAFVGEIMKS	5 h	The peptide was covalently immobilized with EDC-NHS activated 6-mercapto-1-hexanol	22	–	(Dong and Zhao, 2015)
Magainin I	2 h	The peptide was covalently immobilized with EDC-NHS activated 11-mercaptounde-canoic acid	23	α-Helix	(Humblot et al., 2009)
Colicin V	Overnight	The peptide was covalently immobilized with EDC-NHS activated cysteine thiol		α-Helix	(Jiang et al., 2015)
Magainin I	30 min	The peptide was covalently immobilized with EDC-NHS activated 6-mercapto-1-hexanol	23	α-Helix	(Li et al., 2015)
Magainin I	30 min	The peptide was covalently immobilized with EDC-NHS activated ferrocene	23	α-Helix	(Li et al., 2014)
Clavanin A	35 min	The peptide was covalently immobilized with EDC-NHS activated cysteine thiol	37	α-Helix	(Andrade et al., 2015)
Magainin I	Overnight	Amine functionalized glass beads were incubated with mM N-[−maleimidobutyryloxy] succinimide ester subsequently the peptides were immobilized on maleimide-activated beads	23	α-Helix	(Yoo and Lee, 2016)
C16G2cys and G10KHcys	40 min	Cysteine terminated peptides were covalently (directly) immobilized on the surface	–	–	(Lillehoj et al., 2014)
Warnericin RK	1 h	The peptide was covalently immobilized with EDC-NHS activated mercaptohexa decanoic acid	27	α-Helix	(Islam et al., 2020)
hLF1–11	–	The peptide was covalently immobilized with the terminal epoxy group of the silane attached IDEA surface	–	α-Helix	(Hoyos-Nogués et al., 2016)

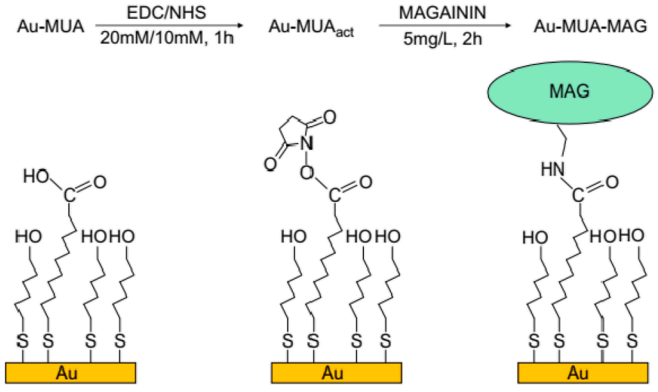

Fig. 4. An overview of the covalently immobilized peptide on biosensor surface, 1st, absorption of thiols on the electrode surface, 2nd, activation of -COOH group presence in thiols by NHS/EDC coupling reagents, 3rd, covalent immobilization of peptide on electrode surface through the activated thiols (Humblot et al., 2009).

due to the chain cleavage reactions, hence the bound AMPs could disassociate from the sensor surfaces (Zoppe et al., 2017).

It is important to note that the behavior of surface functionalized and soluble peptides is significantly different since the surface functionalized peptides are only allowed to interact with bacterial envelopes as they can not enter into the cells (Pardoux et al., 2020). Higher activity of surface functionalized peptides has been reported compared to the soluble counterparts probably due to the high localized concentration (Gao et al., 2012; Yasir et al., 2020). Moreover, less activity in aqueous solution could be attributed to the unfavorable orientation, steric hindrance, reducing dynamics of peptides (Lim et al., 2014; Pardoux et al., 2020). However, further strudies are highly required to elucidate the mechanism of peptide interaction between bacterial envelopes, surface functionalized and soluble conditions.

5. Specificity of AMP towards bacteria

The selectivity or specificity of AMP towards the bacteria plays a vital role for specific detection of pathogenic microbes. Recent studies have shown that the AMP can selectively interact with strain level for certain bacteria as showed in Table 4. It is well known that the AMP can selectively interact with gram positive and gram negative bacteria (Table 6). Bacteria are broadly classified as either Gram-positive or Gram-negative, based on their cell envelopes as presented in Fig. 5.

Table 6
Molecular targets of AMP against Gram-positive and Gram-negative bacteria.

Type of bacteria	Molecular targets
Gram-positive bacteria	- Peptydoglycan - Lipoteichoic acid - Teichoic acid - Lipid II
Gram-negative bacteria	- Lopopolysaccharide

Generally, Gram-positive bacteria contain a crosslinked peptidoglycan layer (PGL) surrounded by various negatively charged molecules (i.e., teichoic acid, lipo teichoic acid) (Li et al., 2017). AMPs are electrostatically interact with these molecules and subsequently diffuse through the nano-sized pores present in PGLs (Li et al., 2017; Malanovic and Lohner, 2016). The PGL is less cross-linked and thinner in the case of Gram-negative bacteria. However, gram-negative bacteria contain an additional membrane known as lipopolysaccharide (LPS) located on the outer part of PGL (Malanovic and Lohner, 2016). The LPS molecules are decorated with a significant amount of negatively charged phosphate groups, resulting in an interaction between AMP and bacterial envelopes (Li et al., 2017). The reactivity of AMPs with Gram-negative and Gram-positive varies due to different atomistic interactions (Malanovic and Lohner, 2016). Thus, few of AMPs such as Lucocin A, C15G2cys reported highly active against Gram-positive bacteria while no or less activity were observed for Gram- negative bacteria (Lillehoj et al., 2014). The molecular targets for Gram-positive and Gram-negative bacteria are listed in Table 6.

In the context of strain level specificity, several factors such as length of AMP sequence, immobilization or orientation of AMPs on the sensor surface, the expression level of man-PTS receptors by bacteria, conformation, and site-specific orientation of peptides could substantially

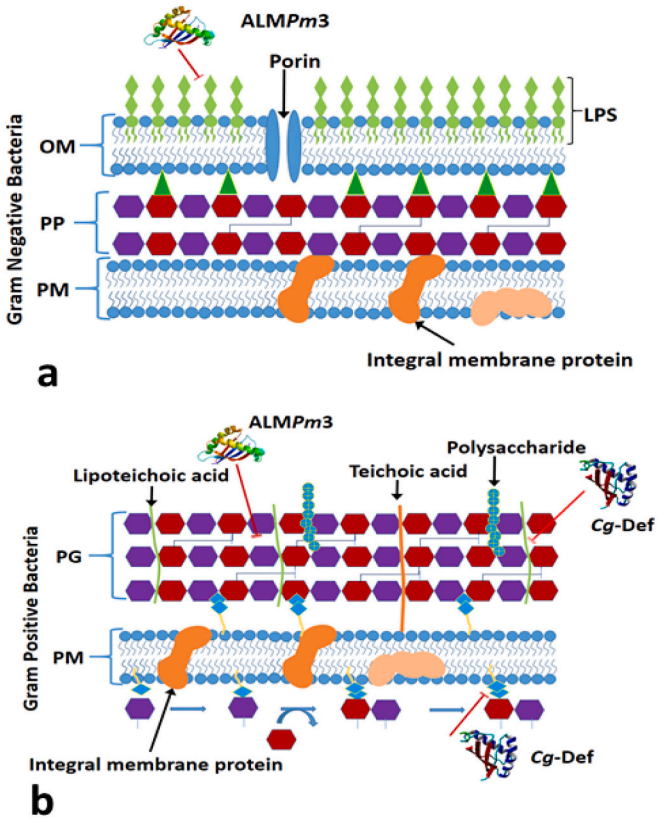

Fig. 5. Schematic bacterial envelope of Gram-negative (a) and Gram-positive (b) bacteria. The plasma membranes are similar for both classes of bacteria. Gram-positive contains a thick peptidoglycan layer while the peptidoglycan in Gram-negative bacteria is comparatively thinner. An additional outer membrane is located surrounding the thin peptidoglycan layer of Gram-negative bacteria.

influence the selectivity of AMPs (Bagheri et al., 2009; Hilpert et al., 2009). Among these factors, the length of the AMP sequence significantly influences affinity towards the bacteria in several ways. Soares et al. (2004) chemically immobilized 6 different lengths and their tailored fragments of AMPs (ceratotoxin A, pleurocidin, PGQ, cecropin P1, cecropin A, and SMAP-29) on a plate to observe the binding affinity against *E. coli*. They found that two tailored fragments obtained significant binding efficiency compared to their complete length of peptides. In other work, the influence of AMP modifications were investigated by assessing the performance of eleven differential fragmented AMPs (Akram et al., 2015). In this study, authors observed that the tailoring had no affinity towards bacteria. Azmi et al. (2015) exposed *L. monocytogenes* to the different fragments of peptide functionalized with gold substrates and found that the high capture affinity of Gram-positive bacteria towards the peptide Lue 10. In the case of leucocin A, both full-length peptide and fragmented peptide (Leu10) demonstrated similar binding affinity to *L. monocytogenes* whereas the other fragments of Leu A displayed a lower affinity for the same bacteria. The authors postulated the binding affinity of peptide fragments varied due to the helical conformation of peptides. Therefore, the length of peptide sequences could substantially influence the affinity of AMPs for capturing bacteria.

In another study, Etayash et al. (2013) reported that the peptide 24 AA Leu A immobilized on a gold surface exhibited differential binding affinities towards several Gram-positive bacteria while no binding variances were observed against a fragment of a similar peptide (14 AA Leu A) as shown in Fig. 6 A-D. This binding variances could be attributed to the short length of peptide, which is not efficient for recognizing the targeted bacterial membrane-bound receptor. In another study conducted by this group (Etayash et al., 2014b), the gold surface was conjugated with AMP 37 Leu A and incubated with 5 different Gram-positive bacteria (*C. divergens, S. aureus, E. faecalis, L. monocytogenes* 19,116 and *L. monocytogenes* 43,256) as shown in Fig. 6B (a-b). Interestingly, a significant binding variance was observed for individual bacteria while pathogenic *L. monocytogenes* showed the highest binding affinity. In addition, they also observed that the C-terminal peptide obtained higher bacteria binding efficiency compared to the N-terminal peptide (LeuA). Besides, the long peptide availed higher bacteria capture efficiency compared to the fragmented length of similar peptide sequence.

In a different study, (Mannoor et al., 2010) also observed bacterial binding variances against AMP magainin I immobilized gold electrode surfaces as shown in Fig. 7A (a-d). In their study, they exposed four different bacteria such as *L. monocytogenes*, *Salmonella typhimurium*, *E. coli* O157, and *E. coli* ATCC 35218, against the AMP functionalized electrode. Interestingly, they observed that *E. coli* O157 obtained the highest bacteria capture efficiency compared to others. However, they could not explain the reasons behind the binding variances of bacteria. Very recently, Islam et al. (2020) reported that peptide warnerecin RK anchored with GaAs semiconductor surface showed attractive specificity against pathogenic *Legionella pneumophila* compared to other bacteria as shown in Fig. 7B (a-g). In this study, the peptide-functionalized GaAs surface was incubated against four different bacteria such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *L. pneumophila* and *E. coli*. They observed that *L. pneumophila* obtained 5 times higher capture efficiency compared to other bacteria. They concluded that the presence of special lipid, namely phosphatidylcholines (30% content), on *Legionella* membrane surface might play a crucial role for higher binding affinity against the peptide warnericin RK (Verdon et al., 2008).

Besides, it has been reported that the orientation of AMPs on the surface of sensor substrates significantly influences the affinity of peptides towards bacteria. Kulagina et al. (2005) compared direct covalent immobilization against indirect covalent immobilization for evaluating capture efficiency of *S. typhimurium* and *E. coli*. The direct covalent method obtained 4 times higher limit of detection in *E. coli* (6.8×10^5 CFU/mL) and *S. typhimurium* (5.6×10^5 CFU/mL) compared to the indirect method for *E. coli* (1.6×10^5 CFU/mL) and *S. typhimurium* (6.5×10^4 CFU/mL). The high performance obtained by the direct covalent method could be attributed to the higher binding affinity of bacteria towards the sensor surface.

Hence, the direct covalent conjugation of the peptide could be considered as an efficient technique to obtain high binding affinity as well as detection sensitivity of bacteria. However, the indirect method is considered more efficient for some materials where the sulfhydryl group cannot be directly functionalized. The helical conformation of immobilized peptides is another crucial factor for achieving high binding affinity. In some studies, it has been reported that the C-terminal peptides showed higher binding affinity than that of N-terminal peptides (Li et al., 2014; Liu et al., 2016). The lower capture efficacy by N-terminal peptide could be due to the lower exposure of amine residues at the vicinity of the N-terminus to the targeted bacteria (Cuervo et al., 1988; Friedrich et al., 1999). Hence, the C-terminal peptide is highly suggested to obtain better binding affinity as well as detection efficiency by the AMP-anchored biosensors.

It is worthy to note that the structure of bacterial membrane, particularly the presence of LPS and the content of their charges, greatly influences the binding affinity of AMPs. In addition, molecular structure of bacterial membrane and absence or presence of antigenic (O-antigen) side chain (Fernandes et al., 2012; Rana et al., 1991) also influence the binding affinity or specificity of peptides towards the bacteria. It has been reported that the number of receptors and composition of bacterial cell membrane also plays a vital role in the specific interaction between bacteria and peptide (Etayash et al., 2013; Vadyvaloo et al., 2004). Recently, Kjos et al. (2009) asserted that the differential expression levels of man-PTS proteins substantially influence the bacteriocin

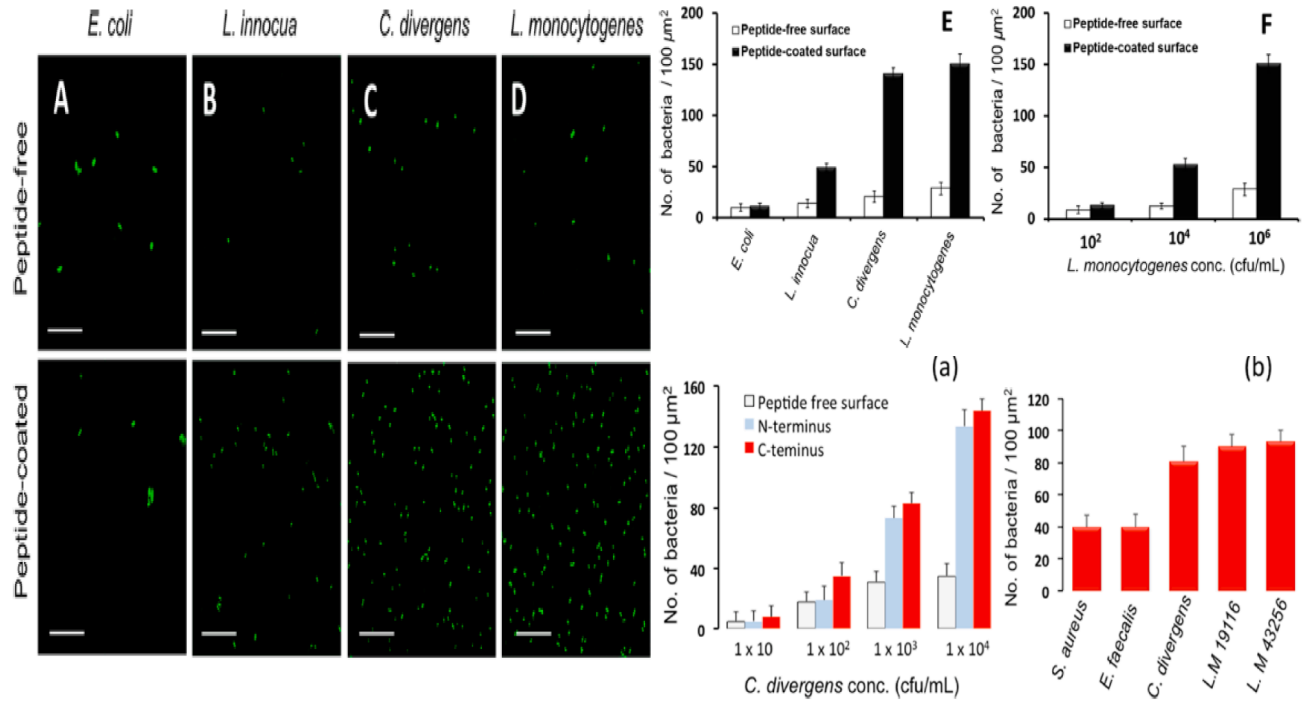

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Fig. 6. The bacteria capture efficiency (average number) of peptide (24AA LeuA) coated and peptide free surfaces (A-F) against different bacteria such as *Listeria innocua*, *Escherichia coli*, *Listeria monocytogenes*, and *Carnobacterium divergens* at 10^6 CFU/mL, the different concentration L. *monocytogenes* (10^2–10^6 CFU/mL) against peptide 24AA LeuA, bacteria capture efficiency (average number) by AMP of LeuA, the *C. divergens* (10^1–10^4 CFU/mL) capture efficiency by the peptide (N or C terminal) coated and uncoated gold surfaces (a), the capture efficiency of C-terminal peptide against different bacteria (*Staphylococcus aureus*, *Enterococcus faecalis C. divergens*, *Listeria. monocytogenes* 191,116, *L. monocytogenes* 43,256) at 10^3 CFU/mL (b) (Etayash et al., 2013; Etayash et al., 2014b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

susceptibility against bacterial cells even within the same species of bacteria. The man-PTS is the main mannose permease in bacteria; however, they also serve as a receptor for subclass IIa bacteriocins as well as subclass IId lactococcin A, and lactococcin B (Tymoszewska et al., 2018). The distinct features of man-PTS plays a crucial role to the bacterial sensitivity aginst bacteriocin peptides (Jeckelmann and Erni, 2020). Generally, bacteriocin (subclass IIa) is known to have a broad spectrum activity against Gram-positive bacteria (i.e., *Streptococcus* spp.,

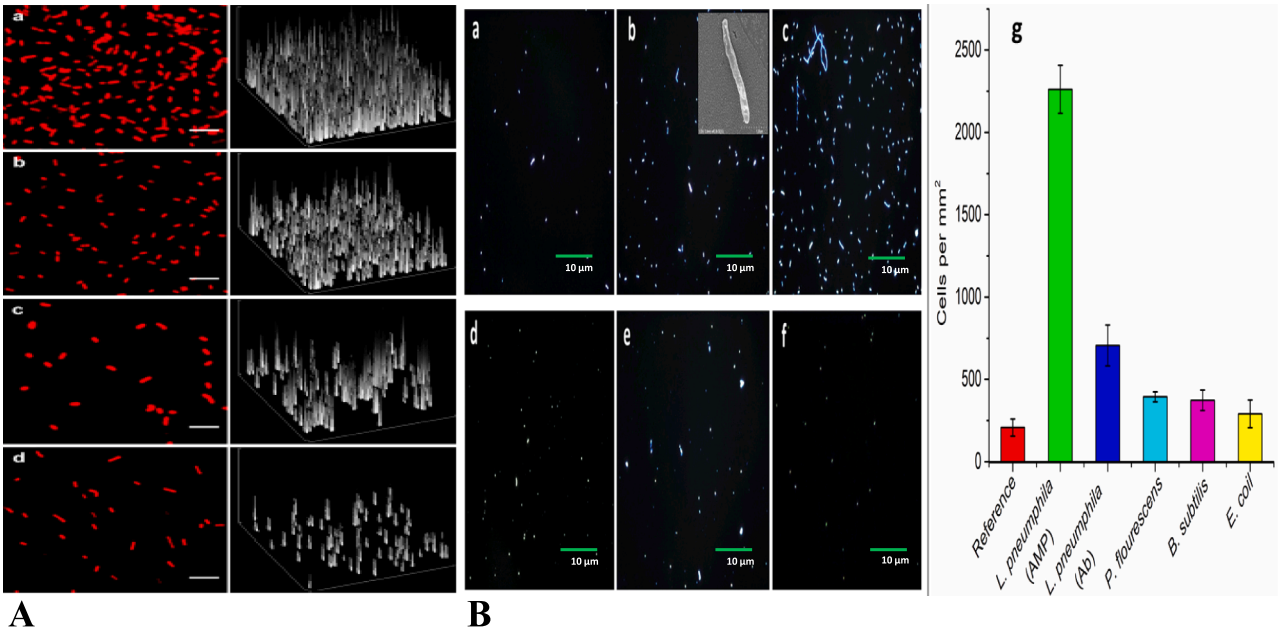

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Fig. 7. A) The selectivity of AMP magainin I against different bacteria (10^7 CFU/mL), a) *Escherichia coli* O157:H7, b) *Salmonella typhimurium*, c) *E. coli* ATCC 35218, and d) *Listeria monocytogenesis*, the scale bar is corresponding to 10 μm; B) the capture efficiency of *Legionella pneumophila* by uncoated surface (a), Ab coated surface (b), AMP coatedsurface (c), and the binding affinity against AMP coated surfaces for (d) *Pseudomonas fluorescens*, (e) *Bacillus subtilis*, and (f) *E. coli*, averaged bacteria surface coverage (g) at a concentration of 10^6 CFU/mL (Islam et al., 2020; Mannoor et al., 2012).

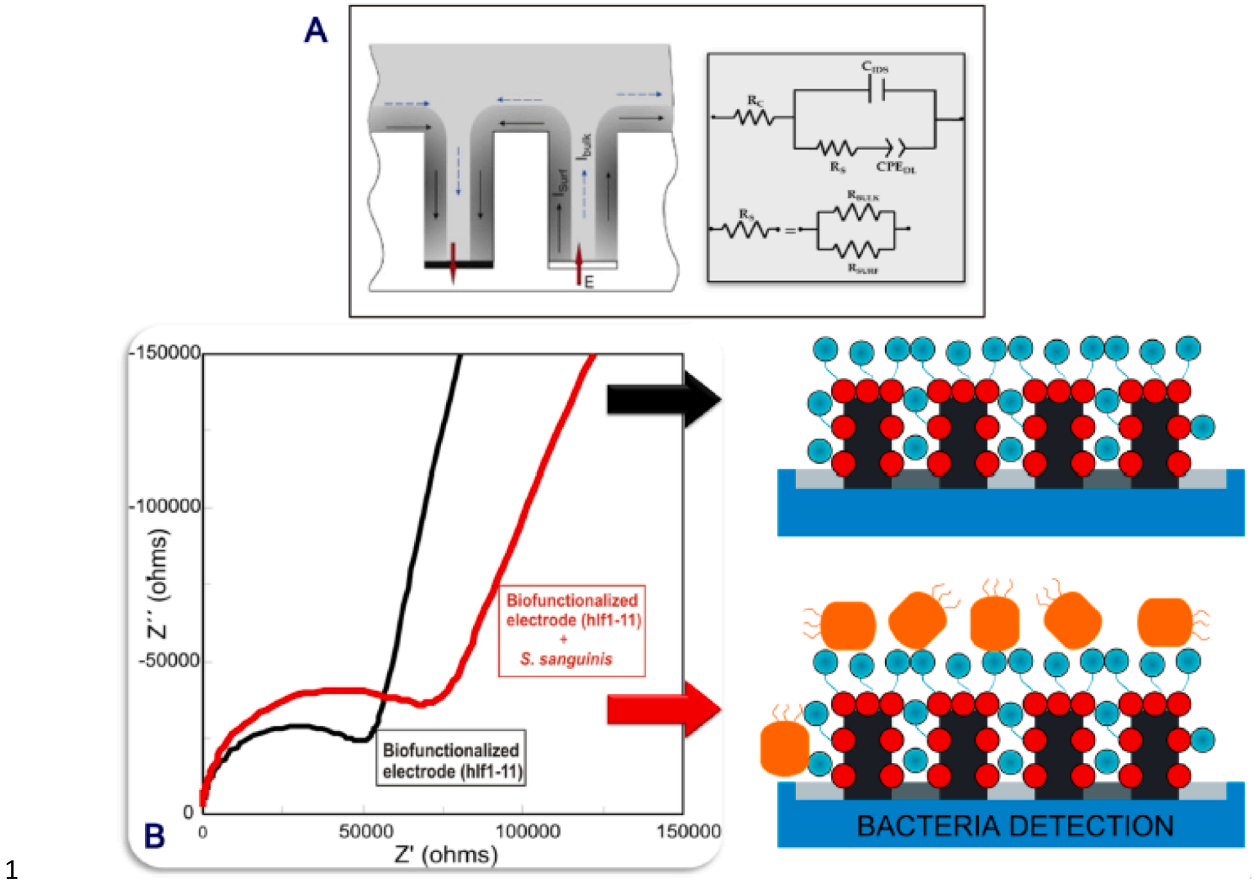

Fig. 8. An overview of the impedimetric biosensor, the electrical circuit used for fitting electrochemical impedance plot (A), the impedance spectrum corresponding to the functionalized gold electrode (black), while the red spectrum is representing the functionalized electrode with the immobilization of *Streptococcus sanguinis*, the schematic representation of electrode functionalization and bacteria capture (B) (Hoyos-Nogués et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Clostridium spp., *Lactobacillus* spp., *Enterococcus* spp., *Leuconostoc* spp., *Pediococcus* spp. and *Listeria* spp.) (Tymoszewska et al., 2018). However, the peptide is mostly active against *Enterococcus* spp. and *Listeria* spp. (Jeckelmann and Erni, 2020). In contrast, LcnA-like bacteriocin shows an extremely narrow spectrum aginst *Lactococcus lactis* in which man-PTS encoded by the *ptnABCD* operon (Tymoszewska et al., 2018).

The PGL is also a key target for the AMPs in the case of Gram-positive bacteria (Malanovic and Lohner, 2016). The Gram-positive bacteria cell envelopes typically show anionic nature due to the presence of teichoic acids and peptidoglycan and thus the cationic AMP creates an electrostatic interaction with the bacterium (Li et al., 2017). In this case, the muramyl peptides, phosphate (i.e., Lipoteichoic and teichoic acids) and carboxyl groups present in the peptidoglycan layer are used as molecular targets for the AMPs as presented in Table 6. In addition, Lipid II has been reported as a potential receptor of AMPs (Malanovic and Lohner, 2016). Wiedemann et al. (2001) observed a specific binding of AMP nisin to the peptidoglycan lipid II precursor of Gram-positive bacteria. In another study, Hasper et al., 2006 reported that the polycyclic peptides (lantibiotics) contain unusual amino acids that target the bacterial cell wall component such as lipid II (Hasper et al., 2006).

It has been revealed that the physiological properties of targeted bacteria could play a crucial role in the antibacterial mechanism against peptide Class IIa bacteriocin peptide (Jacquet et al., 2012). However, the binding variances of bacteria could be regulated by the site-specific orientation and conformation of peptides immobilized on the surface. (Kulagina et al., 2005) observed that the LOD of *Salmonella* sp. and *E. coli* significantly improved while the AMP (magainin I) was uniformly immobilized on the sensor surface. In contrast, it was argued that the sensitivity and selectivity of the biosensor could be significantly decreased, if the bacterial binding receptor-domains are non-uniformly or randomly oriented onto biosensor surfaces (Balamurugan et al., 2008; Jonkheijm et al., 2008). Therefore, afore-mentioned factors should be considered for selecting or designing AMPs to obtain maximum performance by AMPs-anchored sensors.

6. AMPs-based detection of pathogenic bacteria using different transducers

After recognizing bacteria in biosensor moiety, the transducing elements of sensors convert the biological signal into the electrical signals (Hoyos-Nogués et al., 2018). The transducing systems have been categorized according to the type of signal produced by transducers (Ali et al., 2017; Hoyos-Nogués et al., 2018). The transducers could be classified as electrochemical, optical, mechanical, thermal, and sometimes combinations of them (Gaudin, 2017). In the mechanical transducer, the vibrational frequencies of a piezoelectric crystal could be associated with small changes of masses, especially while bacteria are immobilized onto sensors surfaces (Law et al., 2015). However, the electrochemical transducer is widely used that is regulated by the changes of potential and impedance corresponding to the concentrations of targeted analytes (Etayash et al., 2014a). On the other hand, optical transducers are regulated based on light absorbance after exposing light to the biosensor's moiety. The optical transducer allows highly sensitive and specific detection of microbes in a real-time, rapid, and cost-saving manner. Generally, this technique is classified into two groups such as fluorescent label-based and label-free techniques (Lafleur et al., 2016;

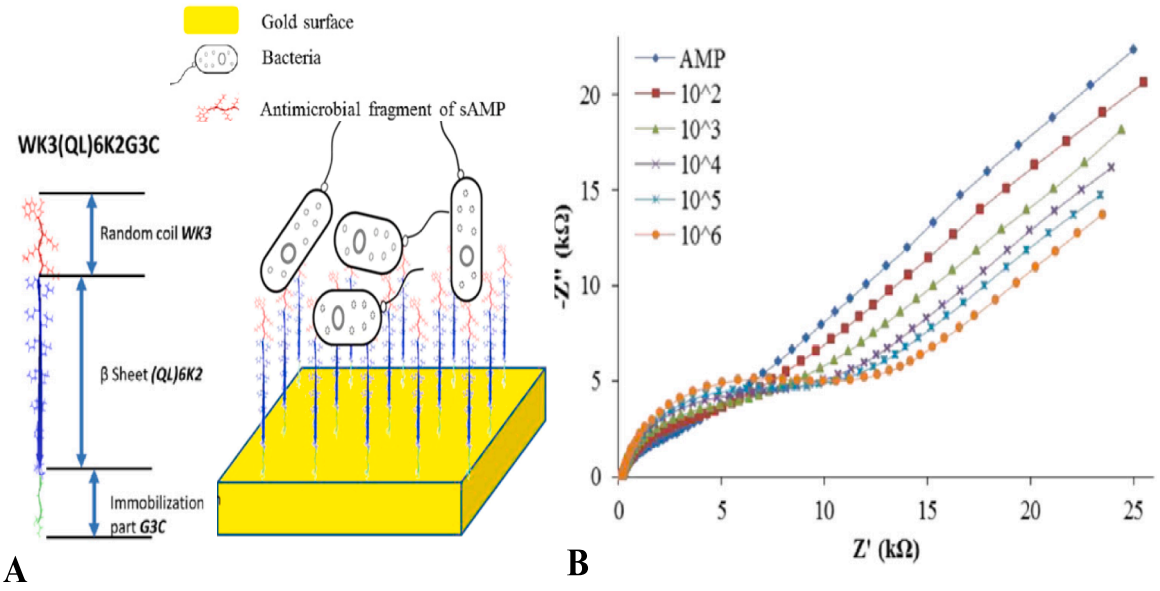

Fig. 9. The cysteine modified AMP functionalization and bacteria attachment on the gold electrode surface (A), Nyquist plot against different concentration (CFU/mL) of *Escherichia coli* (B) (Liu et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Martins et al., 2013). Several types of optical-based sensors such as surface-enhanced Raman spectroscopy (SERS), surface plasmon resonance (SPR), and colorimetric assays have been frequently used for sensing as well as detecting bacteria (Hoyos-Nogués et al., 2018). To date, several transducers such as optical, electrochemical, and piezoelectric have been exploited for detecting pathogenic bacteria for AMP-anchored biosensors as described below.

6.1. Electrochemical transducers

The electro-chemical (EC) is one of the predominant transducing methods for detecting pathogenic bacteria (Lim et al., 2015). In EC transducers, the biological recognition events are sensing in different ways such as a potential, current, and impedance for detecting bacteria. In comparison to other analytical devices, EC detection is robust, inexpensive, fast, and relatively easy to handle. In general, EC transducers comprise three electrodes such as working electrode, counter electrode, and reference electrode. The EC methods can be categorized as amperometric, potentiometric, voltammetric, conductometric, and impedimetric according to the electrical signal generated by the target analytes (Bansod et al., 2017). Among the afore-mentioned EC techniques, the impedimetric transducer is cost-effective as well as easy to handle, since it does not require the formation of electroactive-species (Chauhan et al., 2016). The impedimetric biosensors are mainly regulated by the changes of double-layer capacitance and the resistance of charge or electron transfer between a working electrode and target analytes (Bansod et al., 2017) as presented in Fig. 8. Hence, this technique is considered an efficient method for efficiently recognizing interfacial properties, especially bacteria immobilized with the bioreceptor.

In the impedimetric biosensing technique, generally, bacteria are directly immobilized with an AMP-functionalized electrode. The peptide functionalization allows bacteria to attach at the vicinity of the electrode surface. Thus, the charge discharging properties of bacteria substantially influences the impedimetric signal, which appears as impedance spectroscopy (Sun et al., 2009). Mannoor et al. (2010) designed an AMP (magainin I) based microelectrode impedimetric biosensor to detect Gram-negative bacteria. In their study, they have successfully detected low concentrated pathogenic *E. coli* (10^3 CFU/mL). Interestingly, they observed that the pathogenic *E. coli* generated higher signal compared to the non-pathogenic *E. coli*, which suggested the possibility of strain level bacteria detection using this technique. In another study, Etayash et al. (2014a) used leucocin A, which is known as class IIa bacteriocins to detect Gram-positive bacteria, and they successfully detected *Listeria monocytogenes* at a concentration of 10^3 CFU/mL. Albanese et al. (2019) designed an AMP (nisin) anchored biosensor where they compared binding affinities between N and C terminal peptides, and subsequently, they investigated the detection efficiency of *L. innocua*, *E. coli* O157 by AMP-functionalized electrochemical sensor. In their study, the C-terminus peptide successfully detected low concentrated *L. innocua*. Lillehoj et al. (2014) designed an AMP-anchored impedimetric micro-sensor for detecting pathogenic bacteria, where the sensor detected pathogenic *P. aeruginosa* and *Streptococcus mutans* at a concentration of 10^5 CFU/mL. In another study, Dong and Zhao (2015) developed an AMPs-based impedimetric biosensor for detecting *E. coli* O157. In their study, they effectively detected very low concentrated pathogenic *E. coli* (4×10^2 CFU/mL) in water samples.

Li et al. (2014) designed an AMPs-based ferrocene (Fc) labeled impedimetric biosensor for detecting *E. coli* O157. In their study, Fc was used as a redox probe, which was immobilized on electrode surface through lipoic acid N-hydroxy succinimide, and subsequently, the AMPs were integrated with Fc for capturing bacteria (*E. coli* O157). They observed higher impedance with the reference sample (absence of bacteria) because of improved packing at the interface of solution/peptide. After exposing bacteria to the Fc conjugated AMP film, the impedance was significantly decreased due to the enhanced charge transfer by the Fc group. Their method successfully detected 10^3 CFU/mL of *E. coli* O157 which was highly sensitive compared to the conventional AMPs-based biosensors (LOD = 1×10^4 CFU/mL). However, this technique is considered a complex method since the modification of the electrode is comparatively tedious. Furthermore, there is a possibility of blocking electron transfer due to bacteria captured by electrode surface, which could impact impedance magnitude. Apart from bacteria detection, the AMP-based biosensors can differentiate between dead and live bacteria. In a recent study, Liu et al. (2016) modularly designed cationic multi-domain peptides using an AMP (WK3(QL)6 K2) fragment, specifically integrated on the gold electrode, for detecting and differentiating between dead and live bacteria as shown in Fig. 9. Their technique could successfully detect bacteria such as *E. coli*, *S. aureus*, *P. aeruginosa*, and *Staphylococcus epidermidis* at a minimum concentration of 10^3 CFU/mL along with live and dead bacteria differentiation.

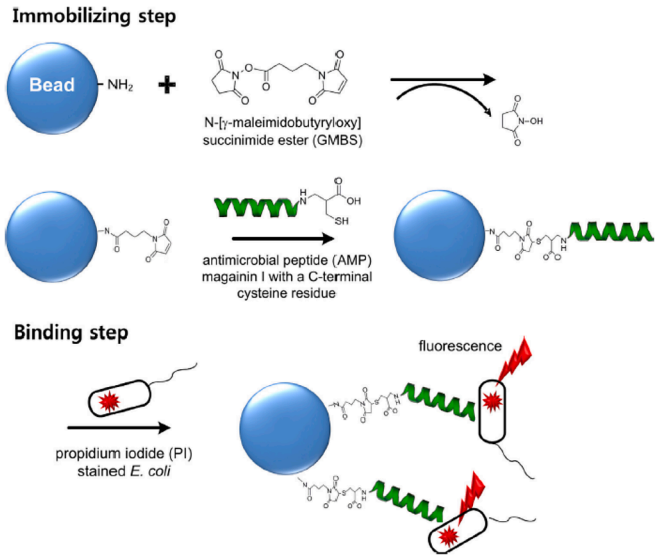

Fig. 10. Schematic presentation of bacteria (*Escherichia coli*) attachment on the AMPs immobilized labeled bead (Yoo et al., 2014).

Apart from impedimetric biosensors, several other electrochemical biosensors, particularly AMPs-based potentiometric biosensors, were also employed for detecting pathogenic bacteria. In potentiometric biosensors, the potential changes are measured as the voltage between the reference and working electrode. In this technique, the concentration of analyte is considered as proportional to the electrical potential, which is compared to a reference potential using a reference electrode. Hence, an accurate and stable reference electrode is required to obtain high selectivity and sensitivity from the sensors. Lv et al. (2018) developed a potentiometric biosensor by anchoring a fragment of Leucocin known as short antimicrobial peptide (sAMP) for detecting *Listeria monocytogenes*. The AMP fragment was integrated with magnetic beads for isolating targeted bacteria from the matrix. Subsequently, captured bacteria were labeled with horseradish peroxidase (HRP) to induce the potential changes on the ion-sensitive electrode via catalyzing substrate. Their proposed technique could detect *L. monocytogenes* in the range of 1.0×10^2 to 1.0×10^6 CFU/mL.

6.2. Optical transducers

The optical transducer detects the changes in light properties due to the interaction between bioreceptors and target analytes. In several recent studies, optical transducers have been widely investigated for bacteria detection due to the high sensitivity, easy to manipulate, and rapid detection response (Rubab et al., 2018). In general, the optical transducers can be classified into three categories, namely fluorescent, colorimetric, and plasmonic techniques as discussed in the subsequent sections (Zeng et al., 2020).

6.2.1. Fluorescent technique

In conventional label-free AMPs-anchored fluorescent techniques, the biosensors substrate are functionalized with AMPs to capture fluorophores dye-stained bacteria. Hossein-Nejad-Ariani et al. (2018a) developed a novel AMP (Leucocin A) based biosensor for specific and rapid detection of *L. monocytogenes*. They successfully detected *L. monocytogenes* at a LOD of 2×10^2 CFU/10 μL using the technique. In another study, Kulagina et al. (2005) detected *Salmonella typhimurium* by using the AMPs-based fluorescent bioassay technique, where they observed similar sensitivity in the detection limit using antibody conjugated biosensor. Subsequently, several AMPs such as cecropin A, polymyxin B, polymyxin E, magainin I, and parasin were chosen to detect *S. typhimurium* and *E. coli O157* (Kulagina et al., 2006).

Interestingly, the afore-mentioned AMPs displayed different binding affinities as well as detection sensitivities for *Salmonella typhimurium* and *E. coli* O157. In their study, they obtained the LODs of 1.0×10^5 to 5.0×10^6 CFU/mL and 5.0×10^4 to 5.0×10^5 CFU/mL for *E. coli* O157 and *S. typhimurium*, respectively. However, their label-free AMPs-anchored fluorescent biosensors could not be considered as a potential technique due to the lower sensitivity (Kulagina et al., 2007).

On the contrary, in labeled-based detection, AMPs are generally conjugated with detection probes (fluorophores) to detect and quantify the targeted bacteria. Arcidiacono et al. (2008) labeled AMPs with Cy5 to detect pathogenic *E. coli* O157 and observed that the detection sensitivity significantly improved using this technique. However, the major constraints of the proposed technique are photo-bleaching and poor stability of organic dyes, which significantly influence the performance of labeled-based fluorescent detection (Lan et al., 2017). Notably, few studies have shown that the quantum dots (QDs) along with wider excitation availed higher stability against photo-bleaching, hence, it is considered as an alternative detection method for organic dyes assisted labeled-based fluorescent detection techniques. Chen et al. (2015) reported that the modification of ZnO QDs using $UBI_{29–41}$ could detect the bacteria in AMP anchored label-based fluorescent technique. In their study, ZnO QDs $UBI_{29–41}$ was covalently linked with each other and they have successfully detected *S. aureus* at a minimal concentration of 1.0×10^4 CFU/mL. However, this technique is capable of detecting only a single bacteria per analysis.

Yoo et al. (2014) fabricated an AMPs-labeled beads micro-channel for capturing the stained *E. coli* as shown in Fig. 10. The fluorescence microscopic technique was used to determine the bacterial concentration. It was observed that the designed bioassay technique could quickly detect pathogenic *E. coli* at a concentration of 10^3 CFU/mL. In another study, J. Han et al. (2017) designed a AMPs/polymer complex bioassay technique for differentiating multiple bacteria. In their technique, they utilized electrostatic interaction between negatively charged poly (paraphenylene-ethynylene) (PPE) and positively charged AMPs, which partly quenched the PPE fluorescence intensity. The PPE-AMP complexes differentially bound on the surface of bacteria, which leads to recognize multiple bacteria at the same time due to the variation of binding affinities to the AMPs. As a result, this technique successfully distinguished fourteen different bacteria in water samples. Therefore, this technique has been considered an effective approach to determine different bacterial species. However, this bioassay technique is not considered appropriate for detecting low concentrated bacteria. Further improvement, especially, signal amplification using innovative nanomaterials is required to enhance the sensitivity of the proposed sensor.

6.2.2. Colorimetric technique

The colorimetric detection method is considered as an attractive optical biosensing tool since the results could be directly observed by naked eyes. In this technique, the detection is determined based on the changes of colour excluding the analytical steps. In general, the AMPs-based colorimetric biosensors could be classified into two categories as enzyme-based and AuNPs based. In enzyme-linked AMPs-based detection, AMPs are generally conjugated with different enzymes (e.g., HRP), acting as a detection probe. Generally, enzyme-AMP conjugation is integrated onto the target bacteria for catalyzing substrates. Consequently, the colour is changed in accordance with the target analytes concentration, which is considered to quantify the pathogenic bacterial concentrations. Qiao et al. (2017a) showed that the low concentrated pathogenic *E. coli* O157 could be detected utilizing HRP-AMPs assisted colorimetric methods, which showed higher sensitivity compared to the conventional antibody-based method. For simplifying the detection process, they further improved the detection technique by utilizing urease-catalyzed signal amplification with the help of magnetic nanoparticles (MNP-AMPs) (Qiao et al., 2017b). In their work, MNPs were conjugated with AMPs to enhance the efficiency of capturing bacteria (*E. coli* O157) by utilizing multiple binding sites of AMP, while for

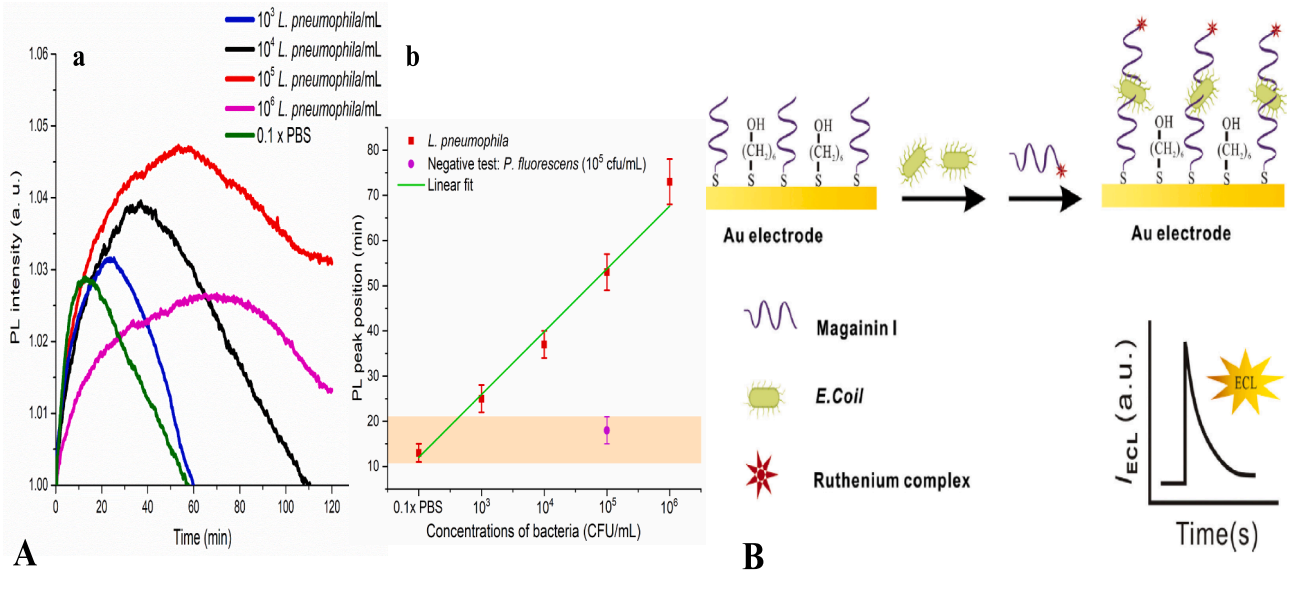

Fig. 11. The normalized PL spectra for AMP functionalized GaAs/AlGaAs semiconductor against different concentrations of *Legionella pneumophila* and PL peak positions vs. bacteria concentrations (A), fabrication of AMP (magainin I) based electrogenerated chemiluminescence (ECL) biosensor (schematic presentation) for the detection of *E. coli* O157 (B) (Islam et al., 2020; Li et al., 2015).

improving specificity, antibodies were inserted with the MNP-AMP conjugation. This bioassay technique allows high capture efficiency of bacteria, with significant enhancement in the signal amplification. Therefore, the immune-magnetic separation assisted AMPs-based colorimetric methods could be a promising approach to detect low concentrated bacteria in a specific way.

6.2.3. *Plasmonic technique*

The SPR and SERS are widely used plasmonic techniques for detecting pathogenic microorganisms. In the plasmonic technique, SPR is an attractive bioassay tool for bacterial detection as it allows real-time detection of pathogenic bacteria. In this technique, the sensors are regulated by the excitation of the electro-magnetic wave, which is spreading through the interfaces of two media (i.e., sample buffer and metal) with differential refractive indices. The AMPs-based bacteria detection platforms can be easily integrated with the SPR transducers via the immobilization of AMPs on the thin film surface. Recently, Zhou et al. (2018) developed AMPs-anchored fiber optic SPR biosensor for detecting pathogenic *E. coli* O157. In their study, they fixed a uniform layer of AgNPs-rGO nano-composite complex by combining silver on the optical fiber for enhancing the SPR signal. Subsequently, the AMP (magainin I) was immobilized on a gold film surface for capturing *E. coli* O157, which induced the wave length shifting of SPR. They successfully detected bacteria ranging from 1.0×10^3 to 5.0×10^7 CFU/mL with a minimum concentration of 5.0×10^2 CFU/mL by utilizing their technique. However, the instrumental cost and complex fabrication process are the main obstacles to design AMPs-based SPR biosensors.

6.3. *Piezoelectric transducers*

Piezoelectric biosensing techniques are another interesting approach for label-free and real-time pathogenic detection. In the piezoelectric biosensing mechanism, the changes of mass on the functionalized electrode induce the change of piezoelectric crystals frequency. Quartz crystal microbalance (QCM) is a commonly known piezoelectric biosensor, where the quartzes are used as piezoelectric material. In a conventional AMPs-anchored piezoelectric biosensor, the QCM electrode is functionalized with AMPs for capturing targeted bacteria in which the mass increases on the surface of the electrode. These changes in mass oscillate the frequency of the crystal, which is considered to determine the concentration of bacteria. Recently, Shi et al. (2017) designed a different type of piezoelectric biosensor, where AMPs dissociate from the electrode surface. In their work, single-walled carbon nanotubes (SWCNTs) were used to modify the electrode surface, and subsequently, the AMPs were immobilized on the SWCNTs modified electrode by utilizing π-π stacking bond. The AMPs attached bacteria dissociate the SWCNTs sidewall from the electrode resulting the shift of piezoelectric sensor frequency, which can be considered to quantify the bacteria. Therefore, piezoelectric biosensors are considered as a promising technique for the determination of pathogenic bacteria; however, the interferences on the surface of the sensor limit the detection sensitivity.

6.4. *Other transducers*

Recently, the photoluminescence (PL) monitored photo corrosion of GaAs/AlGaAs-based detection of target analytes has become popular as it is relatively flexible and could potentially be used to detect analytes in any environmental condition. Furthermore, it is one of the interesting approaches to measure fast optical transitions in semiconductors (Gfroerer, 2000). It is worth mentioning that the intensity of PL strongly depends on the presence of surface-trapped electrically charged molecules. In this way, the measurement of PL is an attractive option for in situ monitoring the presence of negatively charged molecules, such as bacteria immobilized at the semiconductor surface. Very recently, Islam et al. (2020) designed an AMP-anchored DIP PL based GaA/AlGaAs biosensor to detect pathogenic *Legionella pneumophila* as presented in Fig. 11A (a-b). In their study, they exposed different bacteria on AMP-functionalized GaAs/AlGaAs biochips, and interestingly, they found that the peptide-anchored biochip obtained ~5 times higher L. *pneumophila* capture efficiency than the other bacteria investigated. They successfully detected 10^3 CFU/mL *L. pneumophila* through the peptide-anchored GaAs/AlGaAs biosensors.

In a different study, Li et al. (2015) developed an AMP (magainin I) based electrogenerated chemiluminescence (ECL) biosensor for the detection of pathogenic *E. coli* as shown in Fig. 11B. In their study, the magainin I AMPs were covalently immobilized on the gold electrode, and subsequently, the pathogenic *E. coli* O157 was captured on the

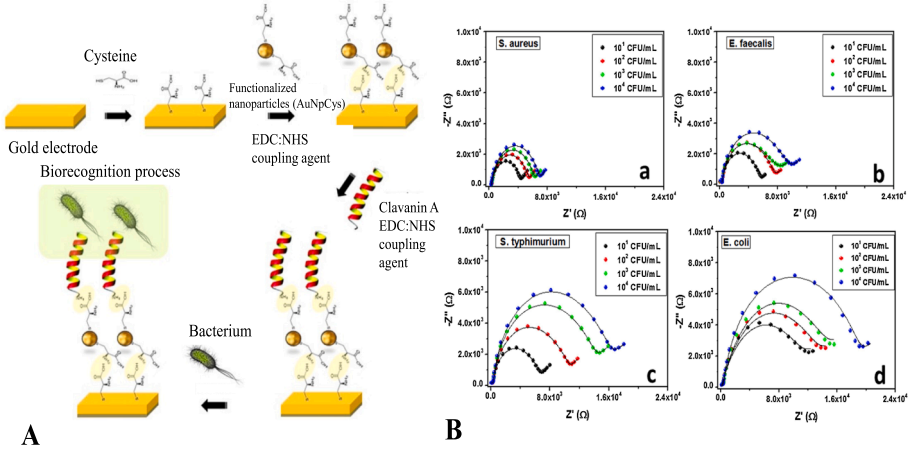

Fig. 12. The schematic representation of AMP immobilization on the gold surface (A), the Nyquist plots obtained from the impedimetric sensor against exposure of different bacteria *Staphylococcus aureus* (a), *Enterococcus faecalis* (b), *Salmonella enterica* (c), and *Escherichia coli* (d) at concentrations ranging from 10^1 to 10^4 CFU/mL (B) (de Miranda et al., 2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electrode surface. In their study, they successfully detected the pathogenic *E. coli* O157 at the concentration ranging from 1.0×10^3 to 5.0×10^5 CFU/mL while the LOD was 2.3×10^2 CFU/mL. Besides, their ECL biosensors could differentiate Gram-negative *E. coli* O157 from Gram-positive bacteria and pathogenic from non-pathogenic *E. coli*.

Another AMP-based bacteria detection technique is known as Lateral flow assay (LFA), which is also an AuNPs-based colorimetric bioassay. Yonekita et al. (2013) developed a multiplex LFA using AMPs labeled with AuNPs for parallel detection of three serogroups of *E. coli*. In their technique, the 3 serogroups were simultaneously captured by antibodies immobilized on the nitrocellulose membrane in different test zones. Meanwhile, targeted bacteria were labeled with AuNPs-functionalized AMPs (AuNPs-AMPs). In this detection, the aggregation of AuNPs induced the formation of red lines at different positions, hence it is a very simple process, and easy to know the bacterial concentration corresponding to the changes of colour during the detection. The LFA multiplex successfully detected *E. coli* bacteria even at the serogroups level at a concentration of 10^4 CFU/mL. However, an enrichment time of 18 h is a prerequisite for this assay, which is the main constraint for this technique to be practically applicable.

6.5. Nanomaterials assisted AMP-based biosensors

In some studies, nanomaterials have been integrated with AMP- for enhancing detection limits as they have unique physicochemical properties along with high mechanical strength. They also substantially enhance the stability and sensitivity of impedimetric biosensors (Kwon et al., 2016; Yang et al., 2015). Andrade et al. (2015) integrated AMPs with carbon nanotubes (CNTs) for enhancing signal transduction in impedimetric biosensors. In their study, CNTs were integrated on the working electrode surface and subsequently, AMPs were covalently linked with CNTs. They observed that the AMP-conjugated CNT captured bacteria could transmit the amplified impedimetric signals. The CNTs-conjugated AMP-based biosensor successfully detected several bacteria at a minimum concentration of 10^2 CFU/mL, which was at least one magnitude lower than studies conducted without CNT-assisted AMPs (Etayash et al., 2014b).

In a recent study, de Miranda et al. (2017) replaced CNTs with gold nanoparticles (AuNPs) since gold can be simply synthesized, and are biocompatible along with high conductivity as presented in Fig. 12. In their work, AuNPs were immobilized on the surface of the electrode and followed by AMPs functionalization on AuNPs to design impedimetric assay. Their method effectively detected differential concentration of bacteria ranging from 10 to 10^4 CFU/mL, which was higher compared to the afore-mentioned AMPs-CNTs-based biosensor. Qiao et al. (2017b) developed an AMP functionalized magnetic nanoparticles.

(AMP-MNP) based fluorescent biosensor for sensitive and rapid detection of *E. coli* O157. In their study, MNPs were conjugated with AMP for capturing *E. coli* O157 due to the hydrophobic and electrostatic interactions. Their proposed biosensing technique could detect *E. coli* O157 with high binding affinity and amplified signal. Hence, integration of nanomaterials with AMPs could substantially improve the detection limit as well as the sensitivity of impedimetric biosensors. However, specially designed nanomaterials, for instance, two or three-dimensional composite nanomaterials, might give a more attractive performance of AMPs-based impedimetric biosensors.

7. Conclusions and future recommendations

Biosensing technique for the detection of pathogenic bacteria is an attractive approach as it allows cost-effective, rapid, sensitive, and specific detection of bacteria. In the past few years, AMP has gained the intention to be used as bioreceptors, as it possesses several advantages (i.e., cost-effective, high stability, and easy to synthesize) over conventional bioreceptors. However, several difficulties need to be considered in designing a potential AMP-based biosensor.

The specificity of AMP is one of the great concerns for detecting certain bacteria because most of the AMP-anchored biosensing techniques have successfully detected a group of bacteria either Gram-negative or Gram-positive. Although several reports have shown that an individual bacterium even at strain level could be detected by AMP-anchored biosensors, still it is a hurdle to determine a specific bacterium in real samples using the interference designed for similar species. Furthermore, it is difficult to design specific detection of certain bacteria as most of the AMPs have shown semi-selective and broad-spectrum natures. However, a better understanding of the peptide-bacteria interaction may help to design an AMP-based biosensor for specific detection of bacteria. Hence, the understanding of the interaction mechanism between the surface of bacteria and AMPs could enhance the possibility to selectively detect bacteria. Besides, the integration of the antibody with AMP and engineering strategies, namely, immunomagnetic separation of nonspecific interaction between bacteria and AMP could be imperative for enhancing the specificity of AMP-anchored biosensors.

The robustness of AMP-anchored biosensors is another concern to make them practically applicable. Up to now, the stability of AMP-anchored biosensors, especially in a harsh environment (e.g., high/low pH, high temperature), has not been studied yet. Most of the studies were conducted in optimal laboratory conditions, hence it is difficult to understand the behavior of sensors dealing with the real samples.

Furthermore, in most cases, the selectivity of AMP anchored biosensors has been investigated by exposing a pure bacterium individually rather than exposing mixed culture bacteria soup. Hence, it is challenging to estimate the selectivity of target bacteria in the ocean of non-target microorganisms. The expected sensitivity of AMP-anchored biosensors is another challenge for considering a potential replacement of existing conventional biosensing methods. As of now, most of the AMP-based biosensors could detect 10^3 CFU/mL of bacteria with few exceptions. However, the dramatic improvement in signal amplification and emerging knowledge of biotechnology, nanotechnology, and microfluidics may improve the detection sensitivity.

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.

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