Chapter 8

Bacterial Detection Using Peptide-Based Platform and Impedance Spectroscopy

Hashem Etayash, Thomas Thundat, and Kamaljit Kaur

Abstract

Antimicrobial peptides have the ability to function as bio-recognition elements in the detection of bacteria. For instance, we showed that Leucocin A, an antimicrobial peptide from class IIa bacteriocins, binds gram-positive *Listeria monocytogenes* with higher affinity than other gram-positive bacteria like *S. aureus*, *L. innocua*, and *E. faecalis*. The binding was detected using impedance spectroscopy when Leucocin A immobilized on impedance electrodes binds bacteria from a sample. Here we highlight the strength of utilizing Leucocin A as a bio-recognition probe in biosensor platforms and provide details on its application in real-time bacterial detection using electrochemical impedance spectroscopy. A simple new generation impedance array analyzer is utilized that works at very low frequencies to identify interactions between peptide and the target bacteria.

Key words Peptide-based biosensors, Antimicrobial peptide, Leucocin A, Impedance spectroscopy, Bacterial detection, *Listeria monocytogenes*

1 Introduction

In recent years, peptides have attracted significant attention as alternative bio-detective elements for developing stable, sensitive and selective sensing devices [1–4]. Their unique properties as substrates for enzymes has enabled the paradigm of different enzyme sensors or enzyme-mediated sensors [5]. Improvement on immobilization and microarray techniques has also enabled the evolution of chip-based peptide biosensors in medical and biological diagnosis [6]. In contrast to antibodies and nucleic acid probes, peptides are naturally more stable, easier to produce or synthesize, and are cheaper. Furthermore, artificial peptides offer an excellent opportunity to develop designer sensors through their desirable properties such as differentiated structures, affinities to enzymes or proteins, ease of synthesis, and use of unnatural amino acids [7].

Ben Prickril and Avraham Rasooly (eds.), *Biosensors and Biodetection: Methods and Protocols, Volume 2: Electrochemical, Bioelectronic, Piezoelectric, Cellular and Molecular Biosensors*, Methods in Molecular Biology, vol. 1572,
DOI 10.1007/978-1-4939-6911-1_8, © Springer Science+Business Media LLC 2017

Impedance spectroscopy is a technique for electrochemical characterization of interfacial processes and is being widely used for the detection of chemical and biological analytes in real-time and label-free mode [8]. The general principle of impedance spectroscopy is based on measuring the dielectric properties of a medium as a function of frequency in AC. In other words, the impedance of a system for a given frequency can be determined from the difference between the phases and amplitudes of the two signals, current and AC potential [9]. In this study, the system uses an affinity based impedance readout to measure any change in the sensor surface due to molecular bindings between the targeted analytes in solution and the immobilized ligands of the sensor interface [1]. This interaction causes changes in the electrical current (AC), which is manifested by measurable changes in the circuit impedance of the electrode double-layer solution interface.

Impedance spectroscopy is a promising sensor platform due to its simplicity, high sensitivity, miniature size, ease of assembling, and flexibility for multiplexed lab-on-a-chip applications. Despite the fact that other biosensors such as microcantilevers [6], nanowire sensors [10], carbon nanotubes [11], and plasmonic sensors [12] can achieve precise detection with high sensitivity, these techniques have not been as simple as the electrochemical impedance spectroscopy. By analyzing the electric property using impedance spectroscopy, information such as the surface charge and molecular conformation change of the analyte can be obtained. It has lately been proven to be a very effective tool for studies including detection of DNA hybridization, antigen–antibody interactions and in the detection of bacterial cells and cancer biomarkers [1, 13–15].

In this chapter, we delineate our effort in developing a peptide-based platform for detection of bacteria using electrochemical impedance spectroscopy. An antimicrobial peptide from class IIa bacteriocin, Leucocin A, was exploited in this study [1]. The peptide expresses extreme activity against pathogenic *Listeria monocytogenes* with minimum inhibitory concentration of ~37 nM [18]. Leucocin A is not only appealing as a safe choice for food preservation, it is also considered as a potential indicator for contaminated food and water [16]. Leucocin A was chemically self-assembled onto gold interdigitated electrodes for electrochemical impedance measurements. A new generation impedance array analyzer (IA-2) that works at very low frequencies was utilized to identify interactions of the peptide to its target bacteria [1, 17].

2 Materials

2.1 Reagents

All reagents and chemical solvents were used as received without any further purification.

1. Fmoc amino acids (Novabiochem).
2. Fmoc−Trp(Boc)−Wang resin (0.2 mmol scale, 0.27 mmol/g) (Novabiochem).
3. 1H-benzotriazolium 1-[bis(dimethylamino)methylene]-5chloro-,hexafluorophosphate (1-),3-oxide (HCTU) (Novabiochem).
4. *N*-methylmorpholine (NMM) (Sigma-Aldrich).
5. Piperidine (Sigma-Aldrich).
6. Trifluoroacetic acid (TFA) (Sigma-Aldrich).
7. Triisopropylsilane (Sigma-Aldrich).
8. Dimethylformamide (DMF) (Caledon Laboratories Ltd).
9. Dichloromethane (DCM) (Caledon Laboratories Ltd).
10. Distillated water.
11. Acetonitrile (Caledon Laboratories Ltd).
12. Ethanol (70%).
13. Phosphate buffered saline (PBS).
14. TSBYE (Tryptic Soy Broth Yeast Extract).
15. APT (All Purpose Tween).

2.2 Equipment

1. Automated peptide synthesizer (Tribute®, Protein Technologies Inc., USA) (*see* **Note 1**).
2. Freeze dryer (Sp-Scientific, Stone Ridge, NY, USA).
3. Reversed-phase High Performance Liquid Chromatography (RP-HPLC, Varian Prostar 210, USA).
4. Matrix Assistance Laser Desorption Ionization-Time Of Flight (MALDI-TOF).
5. UV–visible spectrophotometer (PerkinElmer, Massachusetts, USA).
6. Impedance spectroscopic analyzer SHARP IA-2 (SHARP Laboratories of America, USA).
7. Biosafety cabinet Type II (Microzone Corporation, Ottawa, ON, Canada).
8. Impedance sensor array, Fig. 1a (SHARP Laboratories of America, USA).

2.3 Bacterial Culture

Stocks of bacterial strains were obtained from CanBiocin Edmonton Inc. and were cultivated at 37 °C in an incubator. All

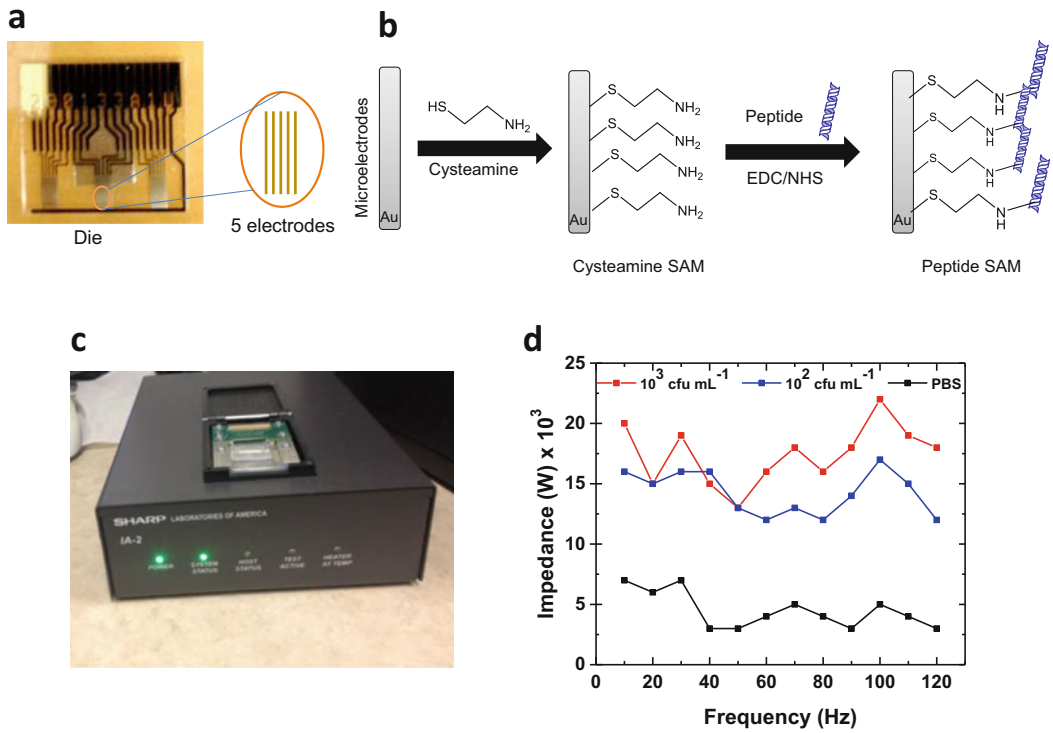

Fig. 1 Peptide functionalization of impedance electrodes. (**a**) An image of the interdigitated gold microelectrode array (Die) of the impedance IA-2 analyzer. (**b**) A schematic representation of the immobilization steps used to make self-assembled monolayer (SAM) of peptide on the gold electrodes. (**c**) The impedance IA-2 from SHARP Laboratories of America. (**d**) Frequency optimization for impedance measurements during bacterial binding to the peptide-conjugated microelectrodes. Reprinted (adapted) with permission from Etayash H, Jiang K, Thundat T, Kaur K (2014) Anal Chem 86:1693–1700. Copyright 2014 American Chemical Society

experiments related to the pathogenic bacteria were carried out in a Type II biosafety cabinet inside a level 2 designated laboratory.

1. *L. monocytogenes* ATCC 43256 (grown in TSBYE, 37 °C).
2. *Listeria innocua* ATCC 33090 (grown in TSBYE, 37 °C).
3. *Enterococcus faecalis* ATTC 19433 (APT broth, 37 °C).
4. *Staphylococcus aureus* ATCC 13565 (APT broth, 37 °C).

3 Methods

All experiments were carried out at room temperature, unless otherwise specified.

3.1 Peptide Synthesis

The antimicrobial peptide, Leucocin A, consists of 37 amino acid residues (NH_2-KYYGNGVHCTKSGCSVNWGEAFSAGVHRLANGGNGFW-COOH), with hydrophobic C-terminal and amphiphilic α-helix structure. The peptide has been synthesized by different methods previously using manual solid phase peptide synthesis

(SPPS) or via an automatic mode of SPPS. We reported synthesis of Leucocin A using native chemical ligation method, where the peptide was separated into two fragments synthesized independently. In the end, the fragments were ligated together in one step to give the full peptide [18]. In this chapter, we describe the synthesis of Leucocin A using SPPS via an automated peptide synthesizer (Tribute®).

1. Fmoc-Trp (Boc)-Wang resin (0.2 mmol scale, 0.27 mmol/g) was initially rinsed with DMF (5 min × 2) and loaded into the Tribute® reaction vessel. The Fmoc-protected amino acids corresponding to Leucocin A sequence were also loaded into the vials (4 equiv).
2. In the Tribute®, the synthesis protocol was set up in order of washing, Fmoc deprotection, washing, and coupling, respectively, for each cycle.
3. Solvents (DMF, DCM), the activating agent (HCTU, 0.8 mM), the base (NMM), and the de-protecting agent (piperidine, 20% in DMF), were placed in their specified flasks, then pressurized and vented as ordered by the manufacture protocol.
4. After complete synthesis, the peptide (37-residues) was cleaved from the resin with removal of all acid-labile side-chain protecting groups by addition of a mixture of 90% TFA, 9% DCM, and 1% triisopropylsilane (~10 mL) for 90 min at room temperature. The cleaved product was collected in a collection tube attached to the Tribute®.
5. The peptide was then precipitated by cold diethyl ether (~15 mL) and collected by further centrifugation. Powdered form of the peptide was obtained via freeze drying of the collected product.
6. Purification of the peptide was carried out on a reversed-phase HPLC, using Vydac semipreparative C18 (1 × 25 cm^2, 5 μm) column. The correct mass $[M+H]^{+1}$ for the pure peptide (reduced) was checked using MALDI-TOF mass spectrometer.
7. Since peptide Leucocin A contains two cysteine residues, the oxidation to form a disulfide bridge between Cys9 and Cys14 was performed by dissolving the peptide in Tris buffer (pH 8.4) at a concentration of 1 mg/mL. DMSO (10%) was used to facilitate the solubility and to expedite the oxidation. The mixture was stirred for 24 h and the oxidized product was subjected to HPLC again to gain pure peptide (*see* **Note 7**).

3.2 Impedance Array (Die) Functionalization

The impedance sensor array (die) consists of three microfluidic reaction chambers with each containing five gold interdigitated gold electrodes (IDEs). The array is fabricated on the surface of

1 mm thick layer of silicon dioxide, deposited on a silicon wafer via plasma enhanced chemical vapor deposition, and was purchased from SHARP Laboratories of America (USA). The typical dimensions of each electrode are 3350 μm × 100 μm × 150 nm and the spacing between each electrode is 40 μm. The size of the die is 20 × 18.5 mm^2 (Fig. 1a).

1. Initially the gold interface washed with Milli-Q water (*see* **Note 6**) of the interdigitated electrodes was coated with a thiolated linker (cysteamine hydrochloride or $HSCH_2CH_2NH_2$) by treating the interface with cysteamine hydrochloride (0.01 M) in 8× PBS buffer solution (pH 8.1). Cysteamine hydrochloride (20 μL, 0.01 M) was added to each of the three microfluidic chambers using a pipettor. After 6 h, each chamber was rinsed with 20 μL of 1× PBS (pH 7.4) to eliminate any unbound cysteamine linker and the washing was repeated twice.
2. Peptide solution (20 μL, 0.8 mg/mL) in 1× PBS (pH 7.4) containing EDC (0.2 M) and NHS (0.1 M) was introduced into the electrodes (chambers) and incubated for 6 h at room temperature. The peptide solution was replaced, and the procedure was repeated once more to ensure complete functionalization of the surface with the peptide (*see* **Note 3**).
3. The functionalized electrodes were then rinsed with 1× PBS to remove any physically adhered molecules.
4. Prior to the experiment, the chambers were rinsed with deionized water, and dried with nitrogen. Figure 1b illustrates the schematic by which the peptide immobilization was performed (*see* **Notes 2** and **4**).

3.3 Bacterial Sample Preparation

All the experiments regarding pathogenic bacteria were carried out in a Type II biosafety cabinet.

1. Stocks of strains of bacterial cells indicated previously, stored at −80 °C in glycerol-supplemented media, were streaked in 1 mL of broth media, APT for *Enterococcus faecalis* ATTC 19433 and *Staphylococcus aureus*, and TSBYE, 37 °C for *Listeria monocytogenes* ATCC 43256 and *Listeria innocua* ATCC 33090.
2. After incubation, the bacterial culture was centrifuged; bacteria were precipitated and resuspended in a phosphate buffered saline—pH 7.4.
3. UV–visible spectrophotometer was used to measure the optical density (OD) at 600 nm and thereafter to calculate the bacterial sample concentrations.
4. The concentration of bacterial cells was obtained from a correlation between the absorbance (OD) and the CFU/mL, based on a calibration experiment.

3.4 Impedance Setup and Optimization

1. The impedance analyzer (Fig. 1c) is capable of measuring the reactions of 15 microfluidic channels simultaneously at a fixed frequency, which can be adjusted at any range between 10 and 1000 Hz, and a stimulation voltage of 10–212 mV. In the experiments, the data presented were performed at 10–120 Hz and 100 mV stimulation voltage.
2. The analyzer is also equipped with a temperature control sensor that can be used to regulate the temperature of the desired reactions. In this study, the temperature was set at 25 °C.
3. The principle by which the IA2 sensor operates relies on the basic principle of the electrochemical impedance spectroscopy. An alteration in the impedance spectrum should occur when the immobilized peptide captures the target bacterial cells. The peptide–bacteria interaction will lead to a change in the impedance parameters that would correlate to the type and amount of the bound bacteria.
4. Initially, we optimized the right frequency for detection by conducting a number of experiments where bacterial solution (20 μL, 10^3 CFU/mL) were introduced into the sensor array and exposed to impedance readings at different frequencies, ranging from 10 to 120 Hz. From these experiments, we noted that a frequency of 100 Hz gives the best impedance response by showing the most stable and the highest impedance magnitude (Fig. 1d). According to the observed results, we designated the frequency at 100 Hz to be the optimum frequency for our detection experiments. Note that in each experiment and prior to the measurement of the peptide–bacteria interaction, the microfluidic chambers of the sensor were incubated with target-free sample (20 μL PBS) first for ~400 s to establish a baseline, then exposed to the contaminated samples and subjected to impedance reading for ~800 s (Fig. 1d) (*see* **Notes 4**, **5**, 7, and **8**).

3.5 Bacterial Detection Using Impedance

1. Sensitivity: To measure sensitivity of the sensor, serially diluted samples contain *Listeria monocytogenes* at 10^2–10^5, and 10^6 CFU/mL was individually injected into the microfluidic chambers (20 μL per chamber), incubated with the immobilized peptide, and subjected to impedance reading for ~800 s at 100 Hz (Fig. 2c).
2. Selectivity: Various strains of bacterial cells at a concentration of 10^3 CFU/mL, including *Listeria monocytogenes* ATCC 43256, *Listeria innocua* ATCC 33090, *Enterococcus faecalis* ATTC 19433, and *Staphylococcus aureus* were introduced into the microfluidic chambers of the impedance sensor and data of the impedance vs. time was recorded in real-time. All experiments were repeated at least three times (Fig. 3) (*see* **Notes 8–10**).

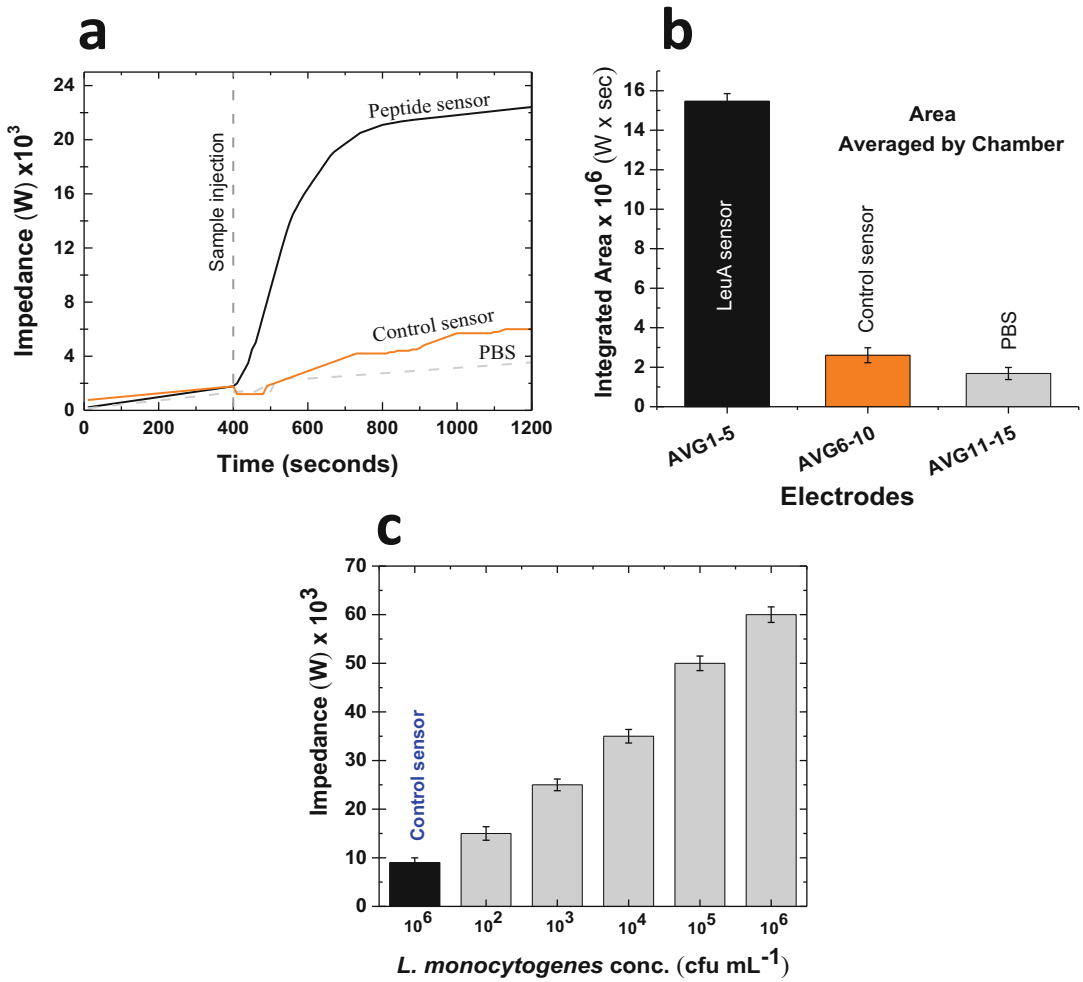

Fig. 2 Peptide-based impedimeric assay for detection of *Listeria monocytogenes*. (**a**) Real-time impedance measurement of *Listeria monocytogenes* interaction to the immobilized antimicrobial peptide at 10^3 cfu/mL, peptide sensor (functionalized with Leucocin A), control sensor (functionalized only with cysteamine), PBS (peptide sensor response to PBS buffer). (**b**) The corresponding integrated area under the curve for *Listeria monocytogenes* interaction to the peptide sensor or control sensor. Each impedance and calculated area is an average calculation of five replicates; *error bars* indicate corresponding standard deviations. (**c**) AMP-coated impedance sensor response to various concentration of *Listeria monocytogenes*, as indicated. Reprinted (adapted) with permission from Etayash H, Jiang K, Thundat T, Kaur K (2014) Anal Chem 86:1693–1700. Copyright 2014 American Chemical Society

3. Regeneration: The impedance sensor regeneration was carried out by confiscating the adhered bacterial cells from the functionalized sensor via washing the microfluidic chambers with 7% alcohol, followed by a rinse with DI water. The study showed that sensor can be regenerated up to 4–5 times (*see* **Note 11**).

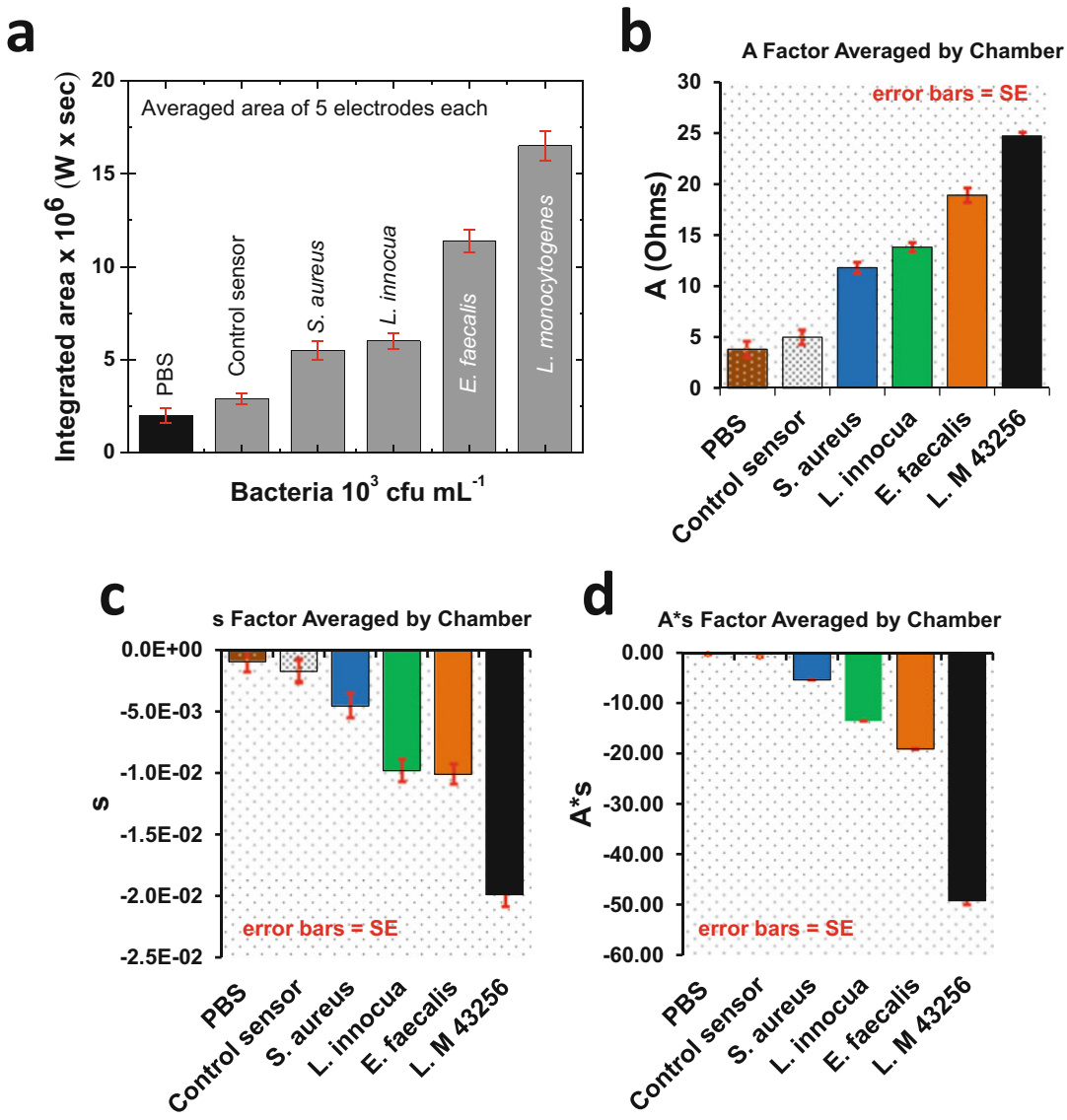

Fig. 3 Selective detection of *Listeria monocytogenes* (L.M) via the Leucocin A-immobilized impedance sensor. (**a**) The binding curve parameters, integrated area under the curve, demonstrates the AMP-based impedance response to various bacterial species at a concentration of 10^3 cfu/mL and fixed frequency 100 Hz. (**b**) Other binding kinetic parameters of the normalized impedance, sensor surface coverage; (**c**) the binding rate constant (s value), and (**d**) the initial rate of binding (A × s) of the peptide–bacteria interaction were analysed as indicated in figures. Results represent an average of five replications and *bars* indicate standard errors. Reprinted (adapted) with permission from Etayash H, Jaing K, Thundat T, Kaur K (2014) Anal Chem 86:1693–1700. Copyright 2014 American Chemical Society

3.6 Data Analysis

The chemistry of the interaction between the immobilized peptide and its target bacteria suggests that the rate of interaction changes with time after injection of the targeted cells. Therefore and according to the equation below, the binding curve at a fixed frequency was analyzed as exponentially varying impedance signal Z over time t.

$$|Z(t)| = \mathrm{B} + \mathrm{A}[1 - \mathrm{e}^{-st}] \quad (1)$$

where s, A, and B are independent constants. B is array impedance baseline of the target free-solution and the offset at starting point of the exponential. The parameter s represents the amplitude of the impedance signal and A is the rate of the exponential time constant that is associated with the target binding. A represents the end point of the binding curve which indicates the density of the sensing layer molecules, size of the analyte particles (bacteria), and dielectric properties of the target molecules. From the above equation an algorithmic extraction of the kinetic parameters is performed automatically and the impedance amplitude is computed consistent to the interaction of the peptide to its target analyte.

The integrated impedance area under the curve is calculated from the starting point of sample injection to the end point where the sample was discharged. The combination of the parameters, $A \times s$, represent the initial rate of binding where the integrated area under the binding curve is dependent upon both A and s, providing characteristics of the target–probe binding behavior.

To conclude, despite a number of limitations that remain to be eliminated, we believe that the concept of peptide-based sensors have a great potential in near future. Peptide molecules as alternative bio-recognition elements can be potentially used for detection of a wide range of analytes, starting from small molecules, metal ions to large cells such as bacteria and cancer cells.

4 Notes

1. The antimicrobial peptide Leucocin A and the negative control peptide were both synthesized using the automated peptide synthesizer, characterized using MALDI-TOF mass spectrometry and purified using RP-HPLC.
2. Peptide immobilization onto the interdigitated gold chip can be performed either via the free carboxylic acids (as shown in Fig. 1b) or amine groups using adequate complementary thiolated linkers. However, as evidenced by a previous report [17], the attachment of the peptide via the carboxylic acid group was more effective in capturing targeted cells.
3. The antimicrobial peptide seems to maintain the alpha helical structure when attached to the impedance electrode surface [20].
4. Numerous surface blocking reagents can be used; however, in our case this was not necessary as it was evidenced by a previous [19, 20] study that it does not do much variation.

5. Impedance optimization is one of the essential steps prior to the experiments. Therefore, it looks like optimization of temperature and frequency is required for each receptor molecule and cell types.
6. Piranha solution reacts violently with sensor electrodes and destroys its reactivity; therefore, it is not recommended at all in this case.
7. Self-assembled monolayer (SAM) of peptide on impedance chips should be used immediately or within a few days. Otherwise, it should be stored in a vacuum desiccator.
8. In the experiment and during exchange samples, the introduction of air into the circulation should be avoided as it causes substantial shift in the baseline.
9. As shown in Fig. 1a, b, the peptide-coated sensor binds well to *Listeria monocytogenes*, causing a dramatic enhancement of impedance resistance compared to its effect against other bacterial strains. The sensor shows high selectivity to *Listeria monocytogenes* (Fig. 3) with detection sensitivity reaching a single cell per μL. The detection limit is recorded as a result of the interpretation of the analyzer and rigorous statistical analysis of the data in comparison to the control sensor. The limit of detection therefore, appears applicable with the clinical standard and compares well with other detection techniques such as antibody-based assay and fluorescence-based tests [21, 22].
10. The kinetic parameters including the area under the curve, the sensor surface coverage *A*, the binding rate constant (s value) and the initial rate of binding (A × s) of the peptide–bacteria interaction (Fig. 3a–d) correlates well with the real time measurements.
11. The impedance array regeneration studies revealed that the sensor is applicable for at least three times of regeneration, however, further investigation are needed. For example, milder condition such as 40–50% ethanol could be more efficient than 70% ethanol. Further, other regeneration reagents can be explored such as glycerol in acidic media could also enhance removal of the cells from the sensor.

Acknowledgments

We acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) for supporting this research. We also thank CanBiocin Edmonton Inc. for providing all strains of bacteria. H. Etayash is the recipient of Alberta Innovates Technology Future Scholarship.

References

1. Etayash H, Jiang K, Thundat T, Kaur K (2014) Impedimetric detection of pathogenic gram-positive bacteria using an antimicrobial peptide from class iia bacteriocins. Anal Chem 86:1693–1700
2. Lim SK, Chen P, Lee FL, Moochhala S, Liedberg B (2015) Peptide-assembled graphene oxide as a fluorescent turn-on sensor for lipopolysaccharide (endotoxin) detection. Anal Chem 87:9408–9412
3. Dong Z-M, Zhao G-C (2015) Label-free detection of pathogenic bacteria via immobilized antimicrobial peptides. Talanta 137:55–61
4. Choi H, Yang Z, Weisshaar JC (2015) Single-cell, real-time detection of oxidative stress induced in Escherichia coli by the antimicrobial peptide CM15. Proc Natl Acad Sci USA 112: E303–E310
5. Yang M, Sun S, Kostov Y, Rasooly A (2011) An automated point-of-care system for immunodetection of staphylococcal enterotoxin B. Anal Biochem 416:74–81
6. Etayash H, Jiang K, Azmi S, Thundat T, Kaur K (2015) Real-time detection of breast cancer cells using peptide-functionalized microcantilever arrays. Sci Rep 5:13967
7. Azmi S, Jiang K, Stiles M, Thundat T, Kaur K (2015) Detection of Listeria monocytogenes with short peptide fragments from class IIa bacteriocins as recognition elements. ACS Comb Sci 17:156–163
8. Zhang XY, Zhou LY, Luo HQ, Li NB (2013) A sensitive and label-free impedimetric biosensor based on an adjunct probe. Anal Chimi Acta 776:11–16
9. Mostowfi F (2007) Electric breakdown of thin liquid films. Ph.D. thesis, University of Alberta, Edmonton, AB, Canada
10. Kang T, Yoo SM, Yoon I, Lee SY, Kim B (2010) Patterned multiplex pathogen DNA detection by Au particle-on-wire SERS sensor. Nano Lett 10:1189–1193
11. Andrade CAS, Nascimento JM, Oliveira IS, de Oliveira CVJ, de Melo CP, Franco OL, Oliveira MDL (2015) Nanostructured sensor based on carbon nanotubes and clavanin A for bacterial detection. Colloids Surf B Biointerfaces 135:833–839
12. Nanduri V, Bhunia AK, Tu S-I, Paoli GC, Brewster JD (2007) SPR biosensor for the detection of L. monocytogenes using phage-displayed antibody. Biosen Bioelectron 23:248–252
13. Ghindilis AL, Smith MW, Messing DS, Haynes VN, Middleton GB, Schwarzkopf KR, Campbell CE, Zhan C, Ulrich B, Frasier MJ et al (2012) Development of real-time assays for impedance-based detection of microbial double-stranded DNA targets: optimization and data analysis. Biosens Bioelectron 35:87–93
14. Messing DS, Ghindilis A, Schwarzkopf K (2011) An improved algorithm for quantifying real-time impedance biosensor signals. Conf Proc IEEE Eng Med Biol Soc 2011:2740–2744
15. Mannoor MS, Zhang S, Link AJ, McAlpine MC (2010) Electrical detection of pathogenic bacteria vis immobilized antimicrobial peptides. Proc Natl Acad Sci USA 107:19207–19212
16. Etayash H, Azmi S, Dangeti R, Kaur K (2015) Peptide bacteriocins—structure activity relationships. Curr Top Med Chem 16:220–241
17. Jiang K, Etayash H, Azmi S, Naicker S, Hassanpourfard M, Shaibani PM, Thakur G, Kaur K, Thundat T (2015) Rapid label-free detection of E. coli using antimicrobial peptide assisted impedance spectroscopy. Anal Methods 7:9744–9748
18. Bodapati KC, Soudy R, Etayash H, Stiles M, Kaur K (2013) Design, synthesis and evaluation of antimicrobial activity of N-terminal modified Leucocin A analogues. Bioorg Med Chem 21:3715–3722
19. Etayash H, Norman L, Thundat T, Stiles M, Kaur K (2014) Surface-conjugated antimicrobial peptide leucocin A displays high binding to pathogenic gram-positive bacteria. ACS Appl Mater Interfaces 6:1131–1138
20. Etayash H, Norman L, Thundat T, Kaur K (2013) Peptide-bacteria interactions using engineered surface-immobilized peptides from class IIa bacteriocins. Langmuir 29:4048–4056
21. Wang J, Xie X, Feng J, Chen JC, Du X-j, Luo J, Lu X, Wang S (2015) Rapid detection of Listeria monocytogenes in milk using confocal micro-Raman spectroscopy and chemometric analysis. Int J Food Microbiol 204:66–74
22. Shi L, Wu F, Wen Y, Zhao F, Xiang J, Ma L (2015) A novel method to detect Listeria monocytogenes via superparamagnetic lateral flow immunoassay. Anal Bioanal Chem 407:529–535