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Development of a rapid and sensitive electrochemical biosensor for detection of human norovirus *via* novel specific binding peptides

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ABSTRACT

Human noroviruses cause acute foodborne gastroenteritis outbreaks worldwide. In this study, a highly sensitive and selective electrochemical biosensor was fabricated for the detection of human norovirus using novel peptides as recognition elements. The electrochemical biosensor was fabricated by assembling of eight novel peptides separately on the gold electrode and investigated their efficiencies for sensing human noroviruses. Among eight peptides, NoroBP peptide coated onto the gold electrode exhibited a high binding affinity towards human noroviruses, resulting a progressive decrease in current signals with increasing concentration of human norovirus (0–10^5 copies/mL). As a result, NoroBP-nonFoul(FlexL)$_2$-coated gold electrode acts an efficient electrochemical biosensor for highly selective detection of human norovirus with a detection limit of 1.7 copies/mL, which is 3-fold lower than the reported methods. The developed electrochemical biosensor was successfully applied to detect human norovirus prepared by standard procedure from oyster, which suggests that the developed biosensor can be used as a very sensitive and selective point-of-care bioanalytical platform for the detection of human norovirus in various food samples.

1. Introduction

Human norovirus belongs to the category of *Caliciviridae viruses*, which are classified into five groups (from GI to GV type) based on the capsid protein sequence and polymerase (Rooney et al., 2014). Among these, GI and GII species have been identified as severe viral species, which cause foodborne illnesses such as irritable bowel syndrome, necrotizing enterocolitis, life-threatening dehydration, and exacerbation of Crohn's disease (Ashiba et al., 2017). It has been shown as a highly infectious virus even at 10^2 copies/mL or fewer virus particles in the human body (Patel et al., 2008; Yoo et al., 2017). Considering their serious causative functions on human health, the development of a facile and simple bioanalytical platform is highly desirable for the identification of human norovirus in various food samples as well as food-processing industries. According to the standard protocol, human norovirus was successfully detected in various food samples, but it highly depends on the laboratory skills and requires several hours to complete analysis of norovirus (Shieh et al., 1999). Further, several molecular biology-based analytical methods such as real-time reverse transcription loop-mediated isothermal amplification (Fukuda et al., 2006), immunochromatographic (Pombubpa and Kittigul, 2012), fluorescence molecular beacon (Adegoke et al., 2016), horseradish peroxidase-integrated polymerase chain reaction (Batule et al., 2018) and enzyme-linked immunosorbent assay (de Bruin et al., 2006) methods have been developed for the detection of human norovirus in various food samples. Although these methods are capable to detect norovirus even at ~10^2 copies/mL, unfortunately they require extensive sample preparation protocols and few issues remain, such as complicated operation, and high-cost, which hamper their wider usability in detecting norovirus in various samples. These emphasize the essentiality for the development of a rapid, simple, and cost-effective analytical platform for the detection of human norovirus with high selectivity and sensitivity.

Recently, significant efforts have been devoted on the fabrication of

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several analytical methods for the detection of human norovirus in various food samples. Briefly, aptamer was incorporated into an electrochemical biosensing platform for sensing of norovirus with enhanced sensitivity (Beier et al., 2014; Giamberardino et al., 2013). An automated microfluidic chip was developed for the detection of norovirus in oyster where the essential procedures such as cell concentration, RNA extraction, amplification, and detection were performed in a chip (Chung et al., 2015). Graphene-gold nanoparticles have been integrated with microfluidic chip for electrochemical detection of norovirus (Chan, Neethirajan, 2017). Similarly, several analytical techniques such as UV–visible spectrometry, field-effect transistor, interferometry, immunochromatographic and electrochemical devices have been used for the detection of norovirus (Auer et al., 2015; Vyas et al., 2015; Xiang et al., 2016; Yakes et al., 2013). Apart from these, lateral-flow assay and pre-emptive electrochemical (Hong et al., 2015) techniques have been developed for immunosensing of norovirus. Among these, electrochemical devices have proven to be simple, portable and low-cost biosensing devices for assaying various biomolecules including norovirus (Grieshaber et al., 2008). It also proved that they provide miniaturized platform for *in-situ* analysis of a wide variety of food-borne pathogens including norovirus (Reta et al., 2018). Conventional electrochemical sensors were measured by using three-electrodes *i.e.,* working electrode, counter electrode and reference electrode. However, these three-electrodes did not fix, which induces different results of experiments due to low reproducibility. To overcome the problem of low reproducibility, screen-printed electrode (SPE) has been introduced by fabricating three-electrodes onto the SPE surface. The SPE exhibited better performance than the conventional three-electrode system with regard to reproducibility and reliability of the electrochemical sensor. To tune the electrode selectivity in cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques, SPE has been modified with nanostructured materials and then decorated with various biomolecules such as peptides and proteins (Geng et al., 2008; Wu et al., 2011). The modified SPE was selectively captured the target biomolecules and pathogens even at ultra-trace levels (Mahmoud et al., 2014; Shu et al., 2013) and improved the analytical performance of electrochemical biosensors with high degree (Cinti and Arduini, 2017; Jo et al., 2017; Li et al., 2017). Furthermore, peptides modified electrodes have proven to be a specific biosensor for the selectivity and sensitive detection of various pathogenic microorganisms (Grieshaber et al., 2008). Since peptides acted as specific recognition elements in electrochemical biosensors because of their structure versatility, specificity and remakrbale binding affinity towards target analytes (Geng et al., 2008; Wu et al., 2011). Here, we report the development and fabrication of electrochemical device with NoroBP-$(FlexL)_2$ peptide as a specific biomolecular binder for the selective and sensitive detection of human norovirus in oyster.

In this work, eight novel noroviral peptides were synergistically assembled onto the surface of gold (Au) electrode separately and evaluated their electrochemical biosensing ability towards human norovirus. To form self-assembly monolayers (SAMs) on the Au electrode surface, the peptides were treated onto the Au electrode separately to form Au-S covalent bond between Au surface and peptide, since peptides have mercapto group (Scheme 1a). Among eight novel noroviral peptides, NoroBP-$(FlexL)_2$ peptide-coated Au electrode exhibited high selectivity to bind with norovirus, resulting to change the electrochemical signal remarkably, which facilitates to develop norovirus sensing device (Scheme 1b). Under the optical conditions, the EIS response of the increases with increasing the concentration of human norovirus. The developed electrochemical device was successfully applied to detect human norovirus in oyster, which confirm the promising practical application of electrochemical biosensor for the detection of norovirus.

2. Experimental

2.1. Chemicals

Fetal bovine serum (FBS), potassium ferricyanide(III), potassium hexacyanoferrate(II) trihydrate and phosphate-buffered saline (PBS, pH 7.4) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Eight novel synthetic peptides (> 95% purity) were received from Peptron Corporation (Daejeon, Korea). Gold screen-printed electrode (Au-SPE) was purchased from EG Technology (C220AT, Seoul, Korea). Dulbecco's modified Eagles medium (DMEM) was obtained from GenDEPOT (Austin, TX, USA).

2.2. Preparation of virus samples

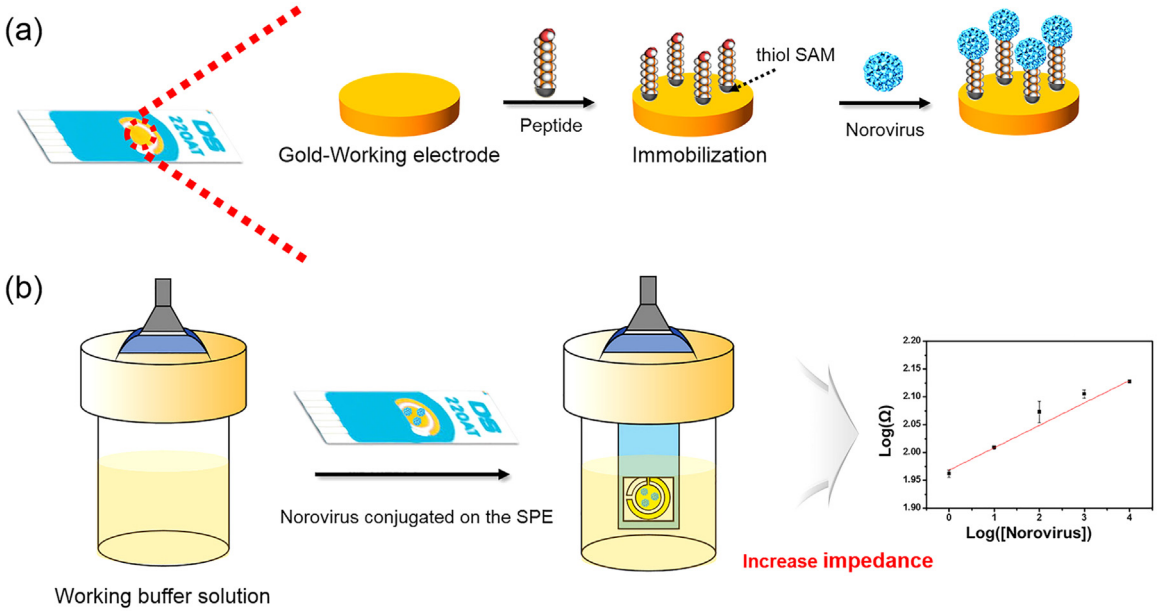
Human norovirus was extracted from norovirus infected humans (Chung-Ang University, Seoul, Korea). To confirm the isolated norovirus, real-time reverse transcription polymerase chain reaction (RT-qPCR) was performed at Chung-Ang University. Based on the observed sequence, the genotype of norovirus is norovirus GII.4 subtype. To be more specific, the human excrement was combined with DMEM (1 − 10) and mixed uniformly by using vortex (Genius 3, IKA, USA) for 5 min. The extracted norovirus was centrifuged at 10,000 × *g*, 4 °C for 15 min. After centrifugation, the supernatant was filtered with 0.8 μm, 0.45 μm and 0.2 μm of the low-protein binding filter (Milipore, Bilierica, MA, USA). The concentration of norovirus was 1×10^6 copies/mL, which was determined by RT-qPCR as per the described procedure in the literature (Lee et al., 2015). Moreover, the human rotavirus was cultured on MA-104 cells (American Type Culture and Collection, Manassas, VA, USA), and these were received from Centers for Disease Control & Prevention (Osong, Korea). The rotavirus culture was carried out as per the protocol in the literature (Seo et al., 2014). Briefly, the rotavirus was extracted by centrifuging with 10,000 × *g*, 4 °C for 5 min. The isolated rotavirus was stored for further use.

2.3. Modification of Au-SPE with novel peptides

The Au-SPE was modified with eight novel peptides separately as the following procedure. First step, the Au-SPE surface was treated with different concentrations of thiol-modified peptides (17 μL; 0.05, 0.1, 0.3, 0.5, 0.7, 1.0, and 2.0 mg/mL) and then incubated at 4 °C for 90 min. After incubation, the Au-SPE was washed two times with ultrapure water to remove unbound peptides. In the second step, 17 μL of different concentrations of norovirus (10^1, 10^2, 10^3, 10^4, and 10^5 copies/mL) was placed onto the peptide-modified Au-SPE and then incubated at 4 °C for 30 min. To remove unbound impurities, the Au-SPE-peptides conjugated norovirus were washed with deionized water, and their electrochemical behaviors were then investigated by EIS techniques.

2.4. Detection of norovirus by using electrochemical analyzer

The electrochemical responses of Au-SPE-novel peptides-norovirus conjugates were examined by EIS and CV techniques. To these analyses, the modified three-electrodes (platinum counter electrode, working electrode and Ag/AgCl reference electrode) were connected to an electrochemical analyzer (CHI 750E, CH Instruments, Austin, TX, USA) and studied their electrochemical behavior. These measurements were pursued in 8 mL of $[Fe(CN)_6]^{3-/4-}$ (25 mM, pH 7.4). The CV was cycled from −0.3 to 0.6 V with a scan rate of 10 mV/s. The EIS was conducted by applying AC potential in the range of 0.09–0.13 V, and the EIS spectra were measured by using the frequency from 100 kHz to 1 Hz.



Scheme 1. Schematic illustration for the detection of norovirus using novel peptide-coated electrochemical biosensor. (a) The novel peptides were immobilized as the SAMs on the Au-working electrode. (b) Using working buffer solution, which can be used for oxidation and reduction. Dropped norovirus on the Au-working electrode was then measured affinity strength by using electrochemical impedance spectroscopy (EIS) analysis.

3. Results and discussion

3.1. Synthesis of affinity peptides and analysis of structural characteristics

As shown in Scheme 1, the electrochemical sensing mechanism is based on the selective interaction of Au-SPE-coated peptides with norovirus. Novel peptides are covalently bonded with the surfaces of Au-SPE, resulting to form synergistic molecular assembly *via* Au-S bond, which facilitates to capture norovirus selectively. For this, high purity (> 95%) cysteine (Cys)- incorporated novel specific binding peptides were synthesized for forming covalent bond with the surfaces of Au-SPE. Noro-1 peptide with amino acid sequence of QHKMHKPHKNTK-GGGGSC was identified by phage display, and the Cys residue was identified at the C-terminus. The Cys-incorporated peptide consists of a linker (-GGGGS-) for molecular flexibility onto the Au-SPE surface, which leads to form SAMs. The NoroBP was used as a scaffold to produce the derivatives of other peptides. The amino acid sequences and characteristics of Cys-incorporated synthetic peptides are shown in Table 1. It was confirmed that the NoroBP is existed with rich acidic amino acids (e.g, Lys). In order to investigate the effects of two repeats of flexible liner on binding interactions, two repeats of a linker (-GGGGS-) were incorporated into NoroBP to form novel NoroBP-$(FlexL)_2$ peptide. The NoroBP-RigidL was prepared by substituting flexible linker with a rigid linker (-EAAAK-), which can be used to study the rigidity of peptides on binding interactions with norovirus. The amino acid sequence is QHKMHKPHKNTKEAAAKC. The NoroBP-$(RigidL)_2$ peptide exists with amino acid sequence of QHKMHKPHKNTKEAAAKEAAAKC, which can be used to study the effect of two repeats of rigid linker on the binding interactions with norovirus. Furthermore, the NoroBP-nonFoul peptide was synthesized by the incorporation of anti-fouling peptide into the NoroBP peptide, favoring for the generation of non-fouling properties of peptides, which impacts on their binding interactions with norovirus. In order to examine the binding nature of peptide with rigidity and non-fouling nature, the NoroBP-nonFoul-$(FlexL)_2$ peptide was synthesized by adding rigid linker and two repeats of flexible linker. In addition, the NoroBP-nonFoul-RigidL peptide was also synthesized to study the combination effect of non-fouling and rigid linker on binding interactions. Finally, the NoroBP-nonFoul-$(RigidL)_2$ peptide was synthesized by incorporation of non-fouling peptide and two repeats of rigid linker, which was used to examine the non-fouling and rigid liner properties on binding interactions. After modifying the novel synthetic peptides with different combinations of linkers, the synthesized peptides were assembled on Au-SPE surfaces and studied their specific binding interactions with norovirus concentration in the range of 10^1- 10^5 copies/mL by EIS (Fig. S1). These results reveal that four peptides, *i.e.,* NoroBP-RigidL, NoroBP-$(RigidL)_2$, NoroBP-nonFoul-RegidL and NoroBP-non-Foul-$(RigidL)_2$ coated on the Au-SPE did not show higher tendency to norovirus since impedance values are not increased with increasing concentration of norovirus. However, NoroBP, NoroBP-$(FlexL)_2$, NoroBP-nonFoul, and NoroBP-nonFoul-$(FlexL)_2$ peptides coated on the Au-SPE exhibited higher tendencies to interact with norovirus even at > 10^3 copies/mL. Based on these observations, the sensitivity and selectivity of four novel peptides (NoroBP, NoroBP-$(FlexL)_2$, NoroBP-

Table 1
The amino acid sequences of synthetic peptides used in this study.

Name	Sequence (N → C terminus)	Note
NoroBP	QHKMHKPHKNTKGGGGSC	Used as scaffold
NoroBP-$(FlexL)_2$	QHKMHKPHKNTKGGGGSGGGGSC	Two repeats of flexible linker
NoroBP-RigidL	QHKMHKPHKNTKEAAAKC	One rigid linker
NoroBP-$(RigidL)_2$	QHKMHKPHKNTKEAAAKEAAAKC	Two repeats of rigid linker
NoroBP-nonFoul	QHKMHKPHKNTKEKEKEKEGGGGSC	Non-fouling
NoroBP-nonFoul-$(FlexL)_2$	QHKMHKPHKNTKEKEKEKEGGGGSGGGGSC	Non-fouling with two repeats of flexible linker
NoroBP-nonFoul-RigidL	QHKMHKPHKNTKEKEKEKEEAAAKC	Non-fouling with rigid linker
NoroBP-nonFoul-$(RigidL)_2$	QHKMHKPHKNTKEKEKEKEEAAAKEAAAKC	Non-fouling with two repeats of rigid linker

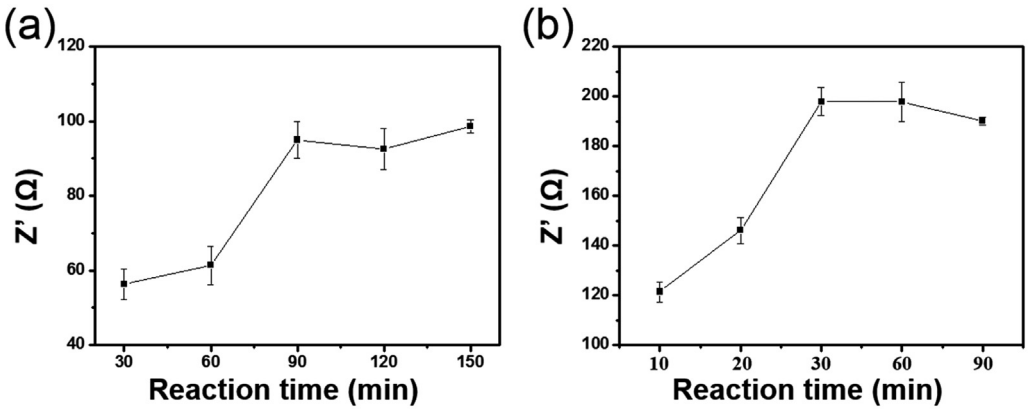


Fig. 1. Optimization of reaction time for the detection of norovirus by using 0.3 mg/mL concentration of NoroBP-nonFoul-$(FlexL)_2$ on the Au-SPE. (a) Optimization of reaction time between the peptide and Au-SPE. (b) Optimization of reaction time between the peptide and norovirus (n = 3).

nonFoul, and NoroBP-nonFoul-$(FlexL)_2$) coated on the Au-SPE were examined.

3.2. Characterization of different novel peptides-SAMs on Au working electrode

The electrochemical behavior of novel peptides coated on the Au-SPE with norovirus was investigated at different reaction time and concentrations of peptides. In order to form effective molecular assembly of peptides on the Au surface, we have studied the effect of reaction time on the impedance values of peptides coated Au electrode with norovirus. As shown in Fig. 1a, the impedance value is gradually increased with increasing reaction time up to 90 min after that the change in impedance is negligible, suggesting that peptides were completely attached on the Au electrode surface. On the contrary, the current value of electrode was decreased with increasing time (Fig. S2). Thus, 90 min was selected as an optimum reaction time for effective molecular assembly of peptides on the Au surfaces with high degree. Then, we also investigated adequate reaction time for binding of peptide-Au SPE with norovirus by EIS. As shown in Fig. 1b, the impedance value gradually was increased with increasing reaction time from 10 to 30 min after that impedance value was slowly decreased. Therefore, we chose 30 min as an optimum reaction time for effective conjugation of norovirus with peptide-modified Au electrode.

Furthermore, the effect of peptide concentration on the Au-SPE surface was investigated for sensitive electrochemical detection of norovirus. Since peptide concentration plays a key role to bind with ultra-trace level norovirus. Importantly, excess peptide concentration leads to form aggregates and double layers on the surface of Au electrode, which impact on the sensitivity of the system (Nahir and Bowden, 1994). Fig. 2 shows the impedance values of Au-SPE with norovirus using different concentrations of four peptides (NoroBP, NoroBP-$(FlexL)_2$, NoroBP-nonFoul, and NoroBP-nonFoul-$(FlexL)_2$) (0.05–2.0 mg/mL). It can be observed that 0.1 mg/mL of peptides (NoroBP, NoroBP-$(FlexL)_2$, and NoroBP-nonFoul) coated on the Au electrodes show better performances whereas NoroBP-nonFoul-$(FlexL)_2$ peptide coated on the Au electrode shows the best impedance value at 0.3 mg/mL. These results confirm that the use of peptides concentrations plays a significant role in electrochemical biosensing of ultra-trace level norovirus. In order to further confirm the role of peptide concentration, we have investigated the effect of NoroBP-nonFoul peptide concentrations (0.05, 0.1 and 0.3 mg/mL) on the Au electrode surface for electrochemical biosensing of norovirus by CV technique (Fig. S3). The best cyclic voltammogram was observed by using 0.1 mg/mL of NoroBP-nonFoul peptide concentration on the Au electrode surface, suggesting that the efficient electron transfer reactions at the interface. The formula for the estimation of current peak using different peptide concentrations was discussed in Supporting information.

3.3. Analytical performance of peptides-coated Au-SPE for norovirus

Under the optimum conditions, we recorded electrochemical impedance spectra of four peptides (NoroBP, NoroBP-$(FlexL)_2$, NoroBP-nonFoul, and NoroBP-nonFoul-$(FlexL)_2$) coated on the Au-SPE with different concentrations of norovirus from 0 to 10^4 copies/mL (Fig. 3). It can be observed that the electrochemical signal intensities of peptides-coated Au electrode were progressively increased with increasing concentration of norovirus (0, 10^1, 10^2, 10^3 and 10^4 copies/mL). The electrochemical impedance spectral data reveals that the distinct changes were observed in the electric current of the device with increasing concentration of norovirus even at 10^1 copies/mL.

In detail, the electrical equivalent circuit model was used to fit the experimental results (Fig. S4). The NoroBP peptide amino acid sequence is “QHKMHKPHKNTKGGGGSC”, which contains cysteine residues for covalent binding of peptides onto the Au-SPE surface *via* Au-S bond as SAM (Hwang et al., 2017). A linker (GGGGS) is used for molecular flexibility, and it is unstructured due to the glycine residues (G), which provide flexibility for molecular assembly on the Au surfaces. The serine residue (S) of linker (GGGGS) has an ability to form hydrogen bonding with the solvents. The NoroBP-$(FlexL)_2$ peptide consists of dimer of $(GGGGS)_2$ linker, and the properties of GGGGS and $(GGGGS)_2$ were reported in the literature (Lu and Feng, 2008; Shan et al., 1999; Venkatesh and Bieniasz, 2013). Figs. 3b and 3d shows the impedance spectra of immobilized NoroBP and $(FlexL)_2$ peptides on the Au-SPE for specific capturing of norovirus and their calibration graphs for the quantification of norovirus. Noticeably, NoroBP-$(FlexL)_2$ peptide coated Au-SPE exhibited higher LOD value (4.97 copies/mL) than the NoroBP peptide coated Au-SPE (2.38 copies/mL). A rigid linker (EAAAK) was inserted into NoroBP peptide, which consists of one glutamate (E), three alanines and one lysine (K) amino acids. The incorporation of this linker into NoroBP peptide can induce the α-helical structure by interrupting the rotation of peptide due to steric hindrance of their methyl group on side chain and stabilized by forming salt bridge (Glu-···Lys+) on the α-helix (Arai . et al., 2001; Marqusee and Baldwin, 1987; Swanson and Sivaramakrishnan, 2014). In addition, alanine (A) residue has methyl group ($-CH_3$) that avoids the interaction with other residues. The other two amino acids (lysine and glutamate) play a key role in forming α-helix structure. In order to enhance the affinity of NoroBP peptide towards norovirus, the nonfouling sequence (EKEKEKE) was inserted into NoroBP peptide. The nonfouling sequence contains four glutamate (E) residues (negative charge) and three lysine (K) residues (positive charge). The EK sequence exists as zwitterions that forms a hydration layer by forming hydrogen bonding onto the Au-SPE. As shown in Figs. 3b and f, the NoroBP-nonFoul peptide-coated

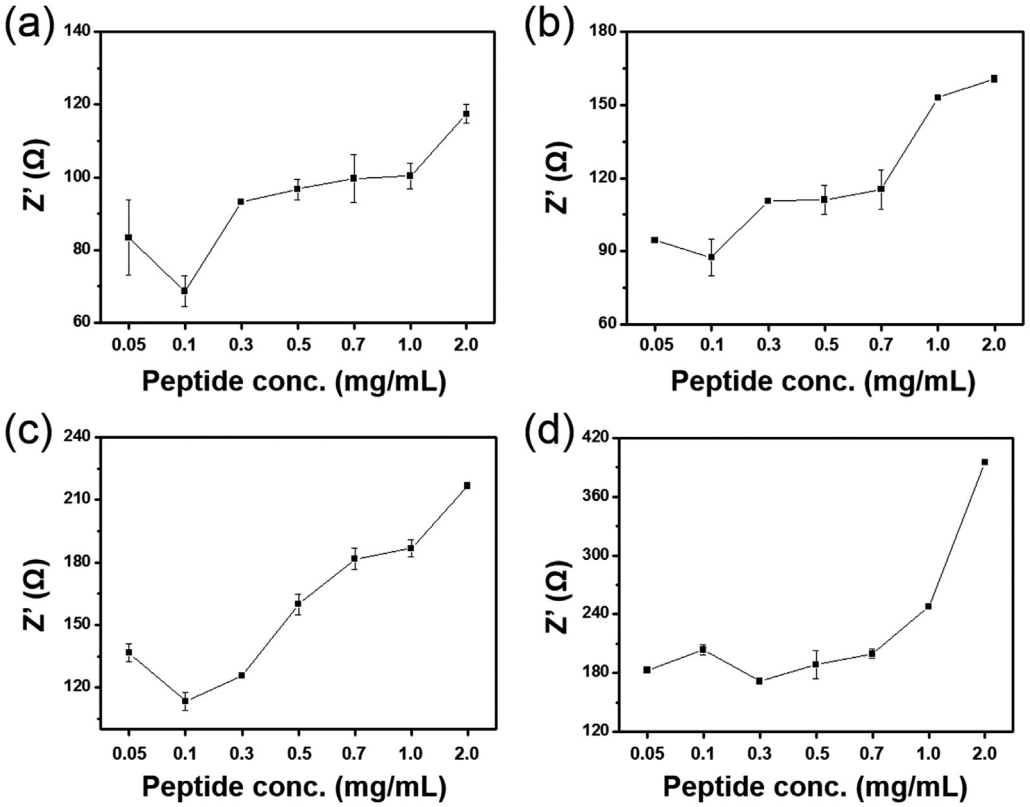


Fig. 2. Optimization of four affinity peptides with different concentrations in order to enhance reaction of specific conjugate peptides with norovirus. (a) NoroBP peptide. (b) NoroBP-$(FlexL)_2$ peptide. (c) NoroBP-nonFoul peptide. (d) NoroBP-nonFoul-$(FlexL)_2$ peptide. (n = 3).

Au-SPE exhibited a lower LOD value (1.99 copies/mL) than the NoroBP peptide (2.38 copies/mL). Based on these observations, $(GGGGS)_2$ and (EAAAK) linkers were inserted into the NoroBP to form NoroBP-nonFoul-$(FlexL)_2$ (EKEKEKEGGGGSGGGGSC), which exhibits the lowest LOD value (1.78 copies/mL). Since nonfouling and flexibility properties of NoroBP peptide were greatly improved by inserting $(GGGGS)_2$ and (EAAAK) linkers, respectively, allowing to capture an ultra-low concentration of noroviruses (Fig. 3h). Interestingly, the NoroBP-nonFoul-RigidL (EKEKEKEEAAAKC) did not show tendency to increase the impedance value with increasing concentration of norovirus (even at $< 10^3$ copies/mL) on the Au-SPE, which this result means that this peptide does not exhibit good analytical performance to detect norovirus at a low concentration ($< 10^3$ copies/mL). Based on the above results, NoroBP-nonFoul-$(FlexL)_2$-coated Au-SPE exhibited the lowest LOD (1.78 copies/mL) for the detection of norovirus, which reveals that the method has exhibited more enough sensitivity to detect norovirus in food samples.

As a result, the EIS signal intensities of four peptides-coated Au-SPE were linear with increasing concentration of norovirus from 0 to 10^5 copies/mL, with regression equations of -Z" (ohm) = $0–10^4 \times \log C$ ($R^2 = 0.9817$), $0–10^4 \times \log C$ ($R^2 = 9848$), $0–10^4 \times \log C$ ($R^2 = 9878$) and $0–10^4 \times \log C$ ($R^2 = 0.9913$) for NoroBP, NoroBP-$(FlexL)_2$, NoroBP-nonFoul, and NoroBP-nonFoul-$(FlexL)_2$ coated on the Au-SPE, respectively (Fig. 3). The detection limits were 2.38, 4.97, 1.99, and 1.78 copies/mL, respectively, using NoroBP, NoroBP-$(FlexL)_2$, NoroBP-nonFoul, and NoroBP-nonFoul-$(FlexL)_2$ coated on the Au-SPE as an electrochemical biosensor. It can be observed that among four peptides-coated Au-SPE electrochemical devices, NoroBP-nonFoul-$(FlexL)_2$ peptide-coated Au-SPE exhibited higher sensitivity to detect norovirus than that of other peptides-coated Au-SPE-based electrochemical biosensors. To explore the analytical merits of developed electrochemical biosensor, the performance of the method was compared with the reported methods on norovirus detection (Table S1). These results confirm that the detection limit of the present method is lower than the other electrochemical biosensors in the literature. To the best of our knowledge, the incorporation of Cys and other linkers into novel peptides sequences and their coating on the Au electrode for electrochemical biosensing of norovirus is described for the first time in this work.

3.4. Evaluation of novel peptides-coated Au-SPE selectivity for norovirus

To test the selectivity of novel peptides-coated Au electrodes towards norovirus, we have investigated the electrochemical impedance spectra of peptides (NoroBP, NoroBP-$(FlexL)_2$, NoroBP-nonFoul, and NoroBP-nonFoul-$(FlexL)_2$)-coated Au electrode with addition of norovirus and rotavirus (1×10^1–1×10^4 copies/mL) (Fig. S5). Since rotavirus also causes gastrointestinal disorders in infants and young children. It can be observed that the addition of various concentrations of rotavirus did not influence to enhance electrochemical impedance spectral intensities. However, the addition of norovirus to peptides-coated Au electrodes, the electrochemical impedance spectral intensities were gradually increased with increasing concentration of norovirus. These results illustrate that the modification of Au electrode with four different novel peptides exhibited high selectivity to detect norovirus, signifying the novel peptides-coated Au electrochemical biosensor for point-of-care detection of norovirus in the real samples.

3.5. Practical application for the detection of norovirus in oyster

In order to explore the practical application of electrochemical biosensor, the NoroBP-nonFoul-$(FlexL)_2$ peptide (0.3 mg/mL)-coated Au electrode was used for the electrochemical detection of norovirus in oyster. Briefly, norovirus was extracted from oyster as per the standard protocol (ISO TS 15271–1). After the extraction of norovirus from oyster, the electrochemical behavior of the NoroBP-nonFoul-$(FlexL)_2$ peptide-coated Au electrode was studied by adding extracted norovirus

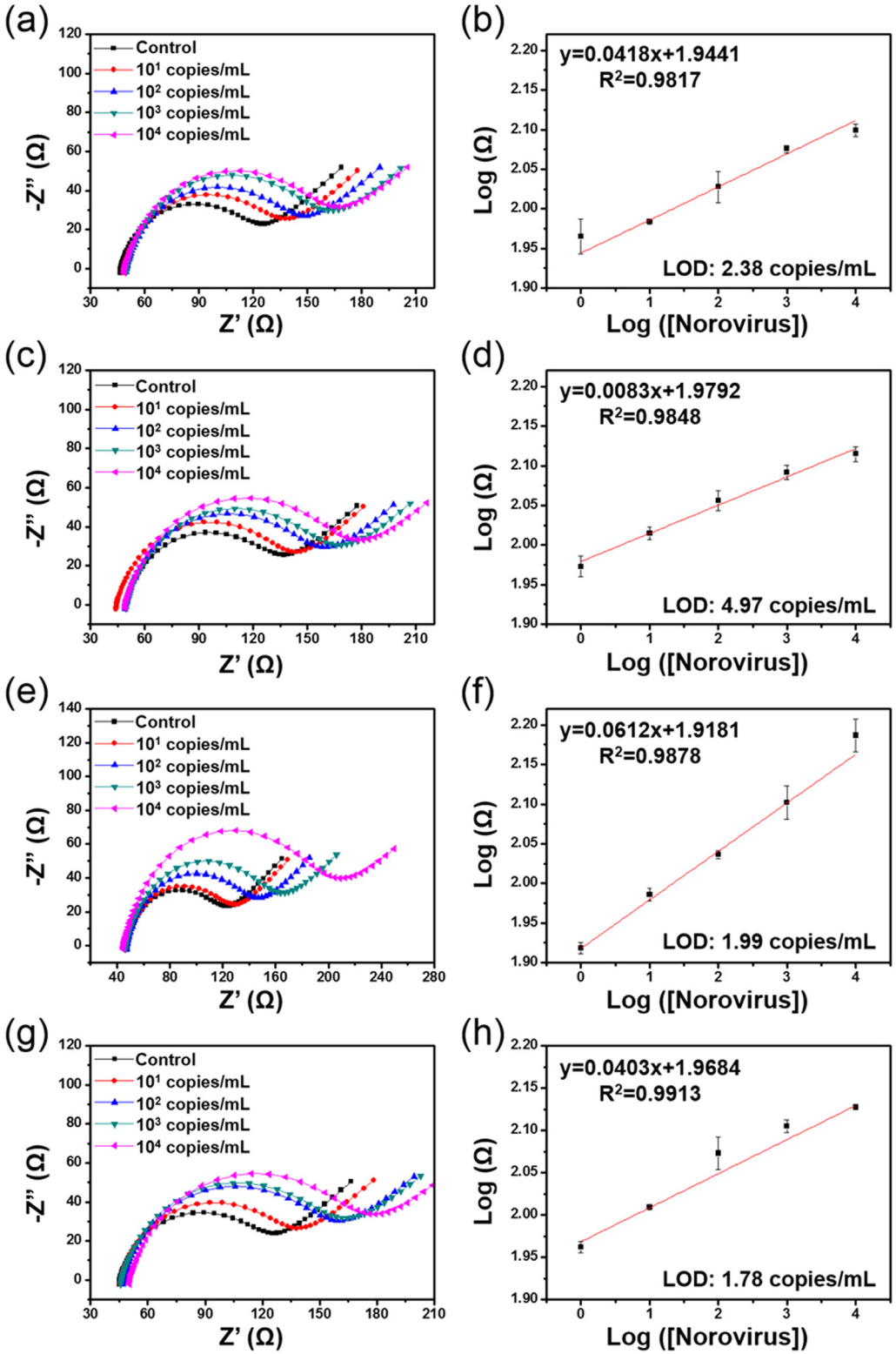


Fig. 3. Electroanalytical performances of four peptides-coated Au-SPE for the detection of norovirus by EIS. (a-e) Electrochemical impedance spectra of different concentrations of norovirus using four peptides-coated Au-SPE. (b-h) Construction of calibration graphs between electrochemical signal intensities of four peptides-coated Au-SPE and different concentrations of norovirus. In detail, (a-b) NoroBP peptide. (c-d) NoroBP-$(FlexL)_2$. (e-f) NoroBP-nonFoul. (g-h) NoroBP-nonFoul-$(FlexL)_2$ (n = 3).

(10^1 to 10^5 copies/mL and 10^0 copies/mL as a negative control) (Fig. S6). It was noticed that impedance signal intensities were progressively increased with increasing concentration of norovirus. The concentration of norovirus was estimated by the standard addition method *via* the construction of calibration graph (Fig. 4). The LOD is 2.47 copies/mL, which is higher than the NoroBP-nonFoul-$(FlexL)_2$ peptide-coated Au electrode-based electrochemical method. This result suggests that the NoroBP-nonFoul-$(FlexL)_2$ peptide-coated Au electrode-based biosensor

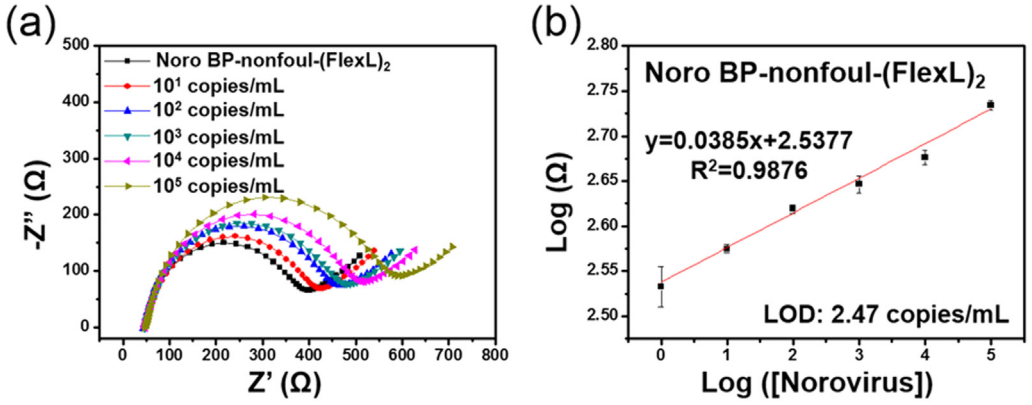


Fig. 4. (a) Electrochemical impedance spectra of NoroBP-nonFoul-$(FlexL)_2$ peptide-coated Au-SPE for the detection of norovirus in oyster by standard addition method. (b) Calibration graph constructed between electrochemical signal intensities and concentration of extracted norovirus in oyster (n = 3).

exhibited selectivity and sensitivity to detect norovirus in oyster, which signifies its potential application in analyzing norovirus in the real samples.

4. Conclusion

In this work, we have introduced eight novel biosensing peptides for the fabrication of Au-SPE and studied their biosensing abilities for ultra-sensitive detection of norovirus in oyster. These novel peptides have provided distinctive behavior on the Au-SPE surfaces, allowing effective capturing of norovirus, which provides to generate a prominent signal response. Among these, four peptides (NoroBP, nonFoul, $(FlexL)_2$, nonFoul-$(FlexL)_2$)-coated Au electrodes acted as promising biosensors for selective and sensitive detection of norovirus. Further, the NoroBP-nonFoul-$(FlexL)_2$ peptide-coated Au-SPE displays high sensitivity for the detection of norovirus, with the low detection limit of 1.7 copies/mL, and good reproducibility. In terms of selectivity and sensitivity, this method has shown outstanding performance than the other molecular biology techniques. This novel peptide coated electrochemical biosensor provides a simple, inexpensive and rapid analytical strategy to detect norovirus in food samples, which offers a promising strategy for identification and quantification of norovirus food contaminants with minimized sample preparations and volumes. Furthermore, these novel peptides coated electrochemical devices may open new horizons for the development of novel biosensors for point-of-care detection of viruses in food samples.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.08.064.

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