Phage based electrochemical detection of *Escherichia coli* in drinking water using affinity reporter probes

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ARTICLE

Phage based electrochemical detection of *Escherichia coli* in drinking water using affinity reporter probes

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The monitoring of drinking water for indicators of fecal contamination is crucial for ensuring a safe supply. In this study, a novel electrochemical method was developed for the rapid and sensitive detection of *Escherichia coli* (*E. coli*) in drinking water. This strategy is based on the use of engineered bacteriophages (phages) to separate and concentrate target *E. coli* when conjugated with magnetic beads and to facilitate the detection by expressing gold binding peptides fused alkaline phosphatase (GBPs-ALP). The fusion protein GBPs-ALP has both the enzymatic activity and the ability to directly bind onto gold surface. This binding-peptide mediated immobilization method provided a novel and simple approach to immobilize proteins on a solid surface, requiring no post-translational modifications. The concentration of *E. coli* was determined by measuring the activity of the ALP on gold electrodes electrochemically using linear sweep voltammetry (LSV). This approach was successfully applied in the detection of *E. coli* in drinking water. We were able to detect 10^5 CFU/mL of *E. coli* within 4 hours. After 9 hours of preincubation, 1 CFU of *E. coli* in 100 mL of drinking water was detected with a total assay time of 12 hours. This approach compares favorably to the current EPA method and has the potential to be applied to detect different bacteria in other food matrices.

Introduction

Safe drinking water is vital to human health and considered a basic human right by the United Nations.[1] Bacteria from fecal sources represent one of the most dangerous contaminants found in drinking water.[2, 3] As an indicator of fecal contamination, "generic" *Escherichia coli* (*E. coli*) is commonly quantified in water sources to determine quality and safety.[4, 5] In order to minimize the safety risk associated with drinking water, an accurate and sensitive detection of *E. coli* is of vital importance. According to the regulations of U.S. Environmental Protection Agency (EPA) on drinking water, there is a "zero tolerance" for "generic" *E. coli*.[6] Therefore, methods designed to determine water quality using "generic" *E. coli* must have a detection limit of 1 CFU / 100 mL. The EPA has approved several methods for the analysis of *E. coli*. Although reliable, traditional culture-based methods rely on selective or differential media which requires days for results.[7] Unfortunately, the delay in determining the water quality without a timely response can result in a risk to public health. Therefore, an easy-to-use, portable and sensitive detection of bacteria with minimal equipment is in need for the water industry.

Electrochemical methods have demonstrated the potential of providing rapid responses with minimal cost and equipment. Compared to other rapid methods, electrochemistry can be applied to detection in turbid or colored samples where the results of colorimetric methods may be less reliable.[8-10] Electrochemistry is an analytical technique used to measure the change of electron transfer caused by a catalytic reaction happens on an electrode or by the change of the electrode surface property.[11] An enzymatic reaction is commonly used to change the electron transfer in which a reporter enzyme catalyzes a substrate to generate an electroactive compound.[12] As reporters in biosensors, enzymes are often used in a sandwich assay and therefore held in proximity to the electrode via the analyte in order to achieve a more sensitive detection. Additionally, several methods of immobilizing enzymes on a solid surface have been proposed, but the widely used methods that involve thiol or silane molecules are based on non-specific adsorption and may cause the immobilization of enzyme in a random orientation.[13, 14]

Binding peptides have emerged as a molecular tool for enzyme immobilization and are a directed and simple method which do not require the need of additional chemicals.[15, 16] Binding peptide tags which are translated along with an enzyme, allow the direct attachment to a solid support.[17, 18] Here, we constructed a fusion reporter consisting of gold binding peptides and alkaline phosphatase (GBPs-ALP), having the enzymatic activity of ALP and the ability to bind with gold surface.

We also utilized bacteriophages (phages) in this assay to facilitate the separation and detection of target *E. coli* from

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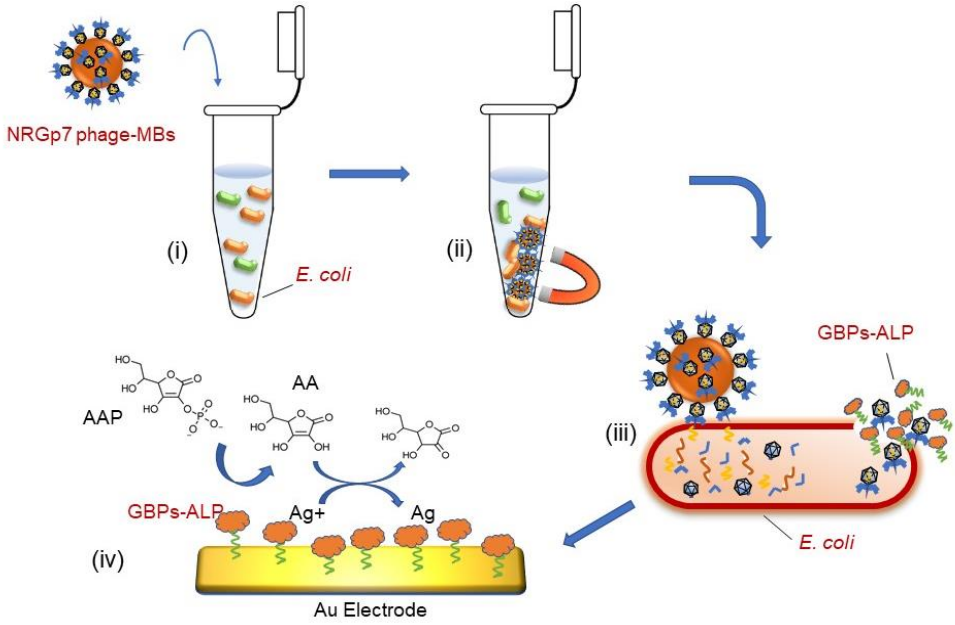

Figure 1: Schematic representation of the engineered-phage based electrochemical detection of *E. coli* in drinking water using binding peptides mediated enzyme immobilization. (i) NRGp7 phage-MBs were added into drinking water (ii)Separation and preconcentration of *E. coli* using NRGp-7 phage-MBs with a magnet (iii) Engineered phage infection of *E. coli* and expression and release of GBPs-ALP (iv) Immobilization of ALP on the gold electrode through the function of GBPs and ALP catalyzed AAP for an electrochemical detection.

drinking water. Phages are virus that can specifically recognize and capture either a broad or narrow range of strains.[19, 20] Phages have previously been used as biorecognition elements to detect bacteria, allowing the advantages of high specificity and the ability to differentiate viable cells.[21-23] Phages can also be conjugated with magnetic beads (MBs) and used for the separation and concentration of target bacteria. In addition, phages can be engineered to carry a gene for a reporter enzyme which can be expressed during viral replication and prior to lysis of the host bacteria.[24, 25] At the end of the infection cycle, phages lyse the bacterial host cells, releasing the replicated phages and reporter enzymes. Several studies have reported the use of phages to facilitate the expression of ALP on the phage surface for enzyme enginneering[26] or free ALP into the solution for bacterial detection.[27] The novelty of our study is the use of phages to express ALP with a specific binding peptide which has the ability to bind onto a solid surface and can facilitate the detection. Our research group have successfully engineered phages to express ALP,[28] beta-galactosidase (β-gal),[29] and tobacco etch virus (TEV) protease.[30] Here, we constructed engineered phages to express the fusion protein GBPs-ALP during the infection of *E. coli*.

In this study, the genetic sequence for GBPs (MHGKTQATSGTIQS) was fused to an ALP gene and inserted into a T7 phage genome resulting in the phage NRGp7. Prior to detection, engineered phages were covalently conjugated onto the surface of MBs for the separation and concentration of *E. coli* from drinking water. Then, GBPs-ALP were expressed and released following completion of the phage infection of *E. coli*. After the immobilization of enzyme ALP on gold electrodes via GBPs, the activity of ALP was quantified electrochemically. The intensity of the electrochemical signal was proportional to the amount of GBPs-ALP expressed from phages, and therefore to the concentration *E. coli* in the water sample. The novelty of this study is that engineered phages were used for both recognition of target bacteria and the expression of reporter protein which could bind a gold electrode (Fig. 1). We proposed a new strategy to immobilize reporter enzymes on a gold electrode using a molecular linker, thereby increasing sensitivity.

Experimental

Chemicals and materials

L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate (AAP), silver nitrate ($AgNO_3$), *N*-hydroxysulfosuccinimide (Sulfo-NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) hydrochloride, and 2-(*N*-morpholino)ethanesulfonic acid (MES) monohydrate, bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Dynabeads M-270 carboxylic acid functionalized magnetic beads were obtained from ThermoFisher (Life Technologies, Warrington, UK). All other analytical grade chemicals were purchased from Fisher Scientific (Fair Lawn, NJ).

All DNA synthesis was provided by IDT (Coralville, Iowa, USA). Phage genomic DNA (T7Select 415-1 DNA) was purchased from EMDMillipore (Burlington, MA, USA), propagated in *E. coli* BL21 and purified for downstream cloning applications as described previously.

Bacterial culture

E. coli (ECOR-13, Thomas S. Whittam STEC center, East Lansing, MI) which was isolated from a healthy human, was grown in Luria-Bertani broth (LB broth, pH 7.4) overnight at 37 °C. The overnight culture was then centrifuged, washed twice with

0.01M phosphate-buffered saline (PBS buffer, pH 7.4) and resuspended in 1mL of PBS buffer. The *E. coli* was then serially diluted into desired concentrations. The concentration of *E. coli* (CFU/mL) was determined by plating on LB agar plates with overnight incubation.

Covalent conjugation of NRGp7 phages on the surface of magnetic beads

Phages were engineered based on standard cloning approaches studied previously and a detailed description is given in Supplementary information.[31] Briefly, a genetic construct of the fusion protein was inserted into the genome of phage directly following the capsid gene. The expression of GBPs-ALP during phage infection of *E. coli* did not significantly affect the infectivity of phages as compared to the wild type. T7 phages carrying a reporter gene for ALP were designated NRGp1 (Accession: MH651795), while T7 carrying a GBP-ALP reporter was designated NRGp7 (Accession: MH703728). NRGp7 phages were then conjugated with magnetic beads (MBs). An aliquot of 100 μL Dynabeads M-270 Carboxylic Acid (~2×10^9 beads/mL, 30 mg/mL) was initially washed with 100 μL of MES buffer (25 mM, pH 5.0) for three times. Immediately before the activation, EDC and sulfo-NHS were dissolved in cold MES buffer to a concentration of 40 mg/mL and 75 mg/mL, respectively. The MBs were then activated in a solution of 50 μL of EDC and 50 μL sulfo-NHS with gentle agitation at room temperature for 30 min. The activated MBs were washed three times with 500 μL of ice-cold 0.01 M PBS buffer, and incubated with NRGp7 phages (1 mL, ~3×10^{10} PFU/mL) overnight at 4 °C. The NRGp7 phage-conjugated MBs (NRGp7-MBs) were then washed five times with PBS buffer, and resuspended in 1 mL of 0.01 M PBS buffer containing 0.1% (w/v) BSA for 2 hours at 4 °C. Finally, NRGp7-MBs were stored in 1 mL of 0.01M PBS buffer at 4 °C for further use. The phage titer (PFU/mL) was determined by standard plaque assay procedure using a double agar overlay. Phages are relatively stable at room temperature but are commonly stored at 4 °C. The NRGp7-MBs were stored at 4 °C and showed similar performance following 2 weeks of storage. For scanning electron microscopy, MBs and *E. coli* were prepared using 2.5% glutaraldehyde to fix overnight at 4 °C. Afterwards, the samples were washed twice with distilled water and dehydrated using serially diluted ethanol solutions. Then the samples were gold sputter coated using a 108 Auto Sputter Coater (TED PELLA, Inc, Redding, CA). The images were obtained using a JCM-6000PLUS NeoScope Benchtop SEM (Peabody, MA) with a voltage of 10 KV.

Electrochemical detection of *E. coli* using engineered phages

Aliquots of *E. coli* (1 mL) with varying concentrations (10^4, 10^5, 10^6, and 10^7 CFU/mL) were independently mixed with 100 μL of NRGp7-MBs for 15 min at room temperature and gentle agitation. The target *E. coli* was separated using a magnet and resuspended in 100 μL of LB broth. Then the NRGp7 phages-MBs-*E. coli* complex was then incubated at 37 °C for 1, 2, or 3 h, allowing for the expression and release of GBPs-ALP. Following incubation, the sample (100 μL) was placed on a screen-printed gold electrode (Dropsens, Asturias, Spain) and incubated for 1.5 hours at 37 °C to allow for enzyme immobilization. After washing off the unbound enzyme, 100 μL of the substrate solution (1 mM AAP and 1mM $AgNO_3$) was placed on the electrode and kept at 37°C for 30 min for the enzymatic reaction. Then 0.6M potassium nitrate (KNO_3) solution (60 μL) was dropped on the electrode, covering all the three electrodes. Linear sweep voltammetry (LSV) from -0.2 V to 0.8 V at 50 mV scan rate was performed to measure the peak current for different concentrations of *E. coli* using a PalmSens potentiostat/galvanostat (PalmSens, Utrecht, Netherlands).

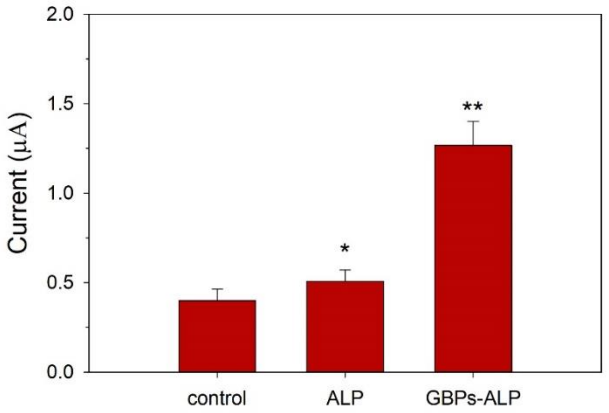

Figure 2: Electrochemical signal obtained from the lysate of phage infections using NRGp1 (ALP), NRGp7 (GBPs-ALP) and control (no phage), respectively. One asterisk (*) represents a significant difference ($0.01 < p < 0.05$) and two asterisks (**) represents a significant difference ($p < 0.01$) between each treated group with the control group. Error bars represent standard deviation of six independent replicates.

Electrochemical detection of *E. coli* in drinking water with pre-incubation step

The drinking water sample was obtained from potable water sources at Cornell University. The water was first treated with sodium thiosulfate tablet (VWR, Radnor, PA) to deactivate the chlorine that might affect *E. coli* growth during the pre-incubation step.[32] *E. coli* cells were serially diluted from the overnight culture to approximate 10 and 100 CFU/mL. Drinking water (100 mL) was inoculated with an aliquot of 100 μL of *E. coli* from these two dilutions to the final concentration of *E. coli* as approximate 1 and 10 CFU per 100 mL, respectively. The concentration of *E. coli* with approximate 100 CFU/mL was confirmed by plate counting as (111.2 ± 62.94) CFU/mL. Then, five times concentrated (5x) LB broth (10 mL) was added into each culture flask for the pre-incubation. The drinking water (100 mL) without inoculated *E. coli* was used as a negative control. All the samples were incubated at 37 °C for 8, 9, and 10 hours. After pre-incubation, the samples were analyzed following the electrochemical detection steps as described in the previous section.

Results and discussion

Evaluation of the performance of GBPs-ALP expressed from engineered phages

The fusion protein GBPs-ALP was evaluated for both the function of GBPs and enzymatic activity of ALP. This bi-functional property was confirmed by the comparison between the GBPs-ALP expressed from NRGp7 phages and the ALP (without GBPs) expressed from NRGp1 phages. Aliquots of 100 µL GBPs-ALP and ALP with the same original enzymatic activity were deposited on gold electrodes, respectively. After the enzyme immobilization and washing off the unbound ALP, the enzyme activities of the GBPs-ALP and ALP remaining on gold electrodes were determined electrochemically.

The immobilization of enzymes on a solid surface with high affinity and material specificity is critical for a wide range of uses, including biosensor development. Binding peptides as an alternative molecular tool for enzyme immobilization, have shown the potential to minimize the problems associated with non-specific adsorption of commonly used enzyme immobilization methods.[18, 33] The GBPs-ALP expressed from *E. coli* have been shown to have oriented immobilization of ALP on gold surface using GBPs as the molecular linker.[17] To our knowledge, our study is the first time to genetically modify phages to express GBPs-reporter enzymes for bacterial detection. As seen from Fig. 2, the electrochemical signal obtained using *E. coli* infection lysate from NRGp7 (GBPs-ALP) was significantly higher than that using NRGp1 (ALP) or control (only LB broth). This suggests GBPs-ALP was able to be immobilized on gold surface while the ALP without binding peptides were removed during the washing steps. Meanwhile, the immobilized fusion protein maintained the necessary enzyme activity to catalyze a reaction and provide an electrochemical signal. Although the ALP without binding peptides did have a measurable difference as compared to the control, this effect is most likely due to non-specific binding. The results confirmed the bi-functionality of GBPs-ALP expressed from engineered phages and demonstrated the effectiveness of using GBPs as a molecular linker genetically fused to ALP for enzyme immobilization on gold surface.

Optimization of the binding condition of GBPs-ALP on gold electrodes

After the evaluation of the bi-functionality of GBPs-ALP, it was necessary to investigate the binding efficiency of the fusion protein at different conditions because a high immobilization efficiency of the enzyme on the gold electrode is crucial for a low detection limit. The immobilization conditions with respect to temperature and time were investigated. GBPs-ALP expressed from engineered phages were placed on the gold electrode and incubated at different conditions followed by the electrochemical measurement. The effect of the temperature on the binding efficiency was studied between 37 °C and room temperature (22 °C). Although the optimal temperature for *E. coli* growth is 37 °C, and the binding peptides were originally selected from the peptides expressed from *E. coli*,[34] room temperature would be more convenient for rapid and low-cost assays. The binding efficiency was also determined using 10 min to 120 min of incubation time.

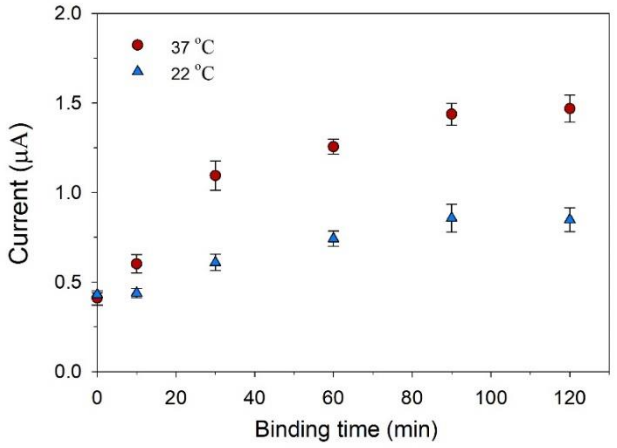

Figure 3: Optimization of the binding condition of GBPs-ALP onto the gold electrode. Dependence of the electrochemical signal on increased binding time (0, 10, 30, 60, 90, and 120 min) at 37 °C (red circle) and 22 °C (blue triangle), respectively. Error bars represent standard deviation of six independent replicates.

As depicted in Fig. 3, the electrochemical signal was determined using initial incubation temperatures of 37 °C and 22 °C with varying incubation times (10, 30, 60, 90, and 120 min). Within the same binding time, a significant higher electrochemical signal was observed when the GBPs-ALP was immobilized at 37 °C than that at 22 °C, suggesting that these binding peptides have a higher binding efficiency at 37 °C. This is in agreement with the original selection conditions of the GBPs.[34] It was therefore necessary to perform the binding procedure at 37 °C for a higher signal. As for the binding time, the results demonstrated that the signal increased gradually with the

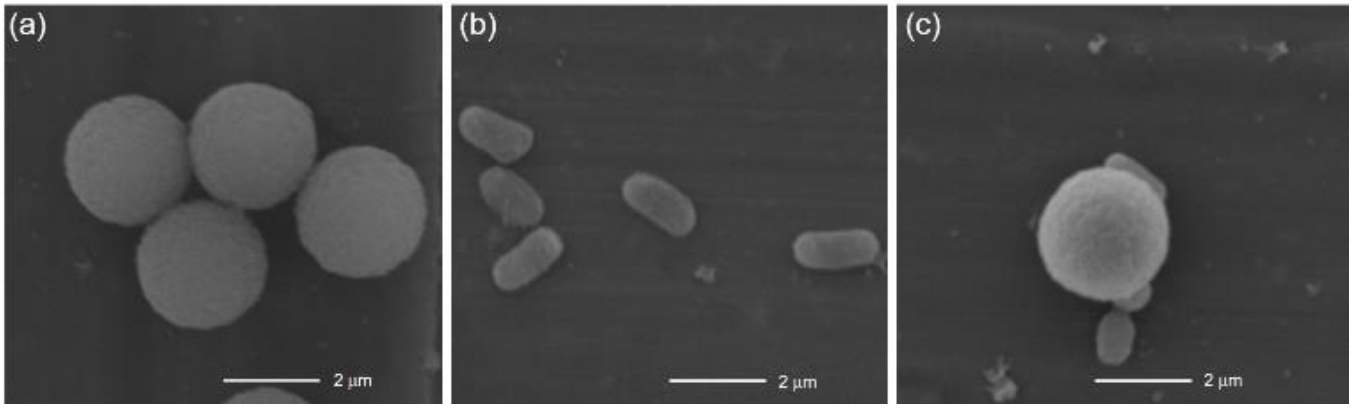

Figure 4: Scanning electron micrographs of (a) NRGp7 phages conjugated magnetic beads, (b) *E. coli* cells, and (c) *E. coli* cells captured by NRGp7 phages conjugated magnetic beads.

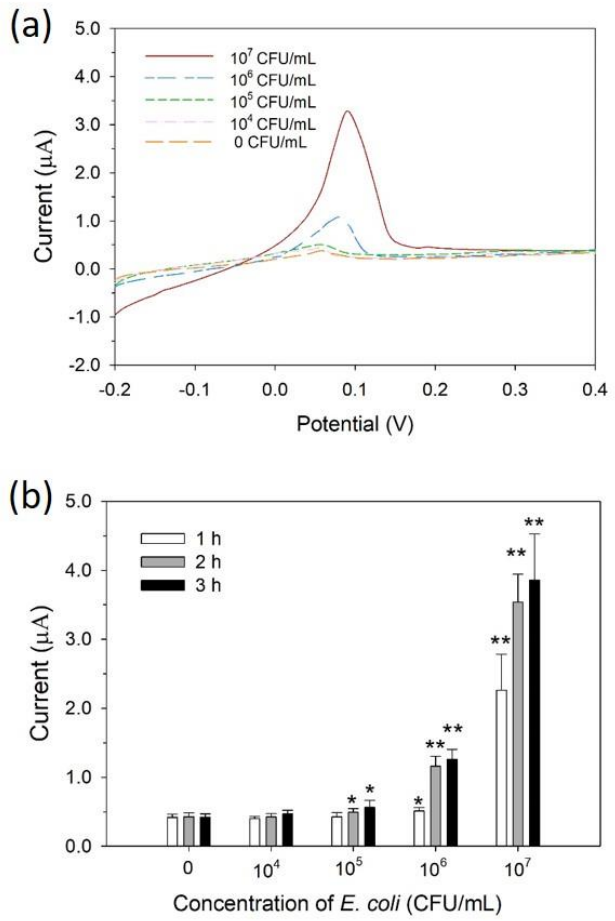

Figure 5: Results of electrochemical detection of *E. coli* using NRGp7 phages. (a) LSV curves for increasing concentration of *E. coli* (0, 10^4, 10^5, 10^6, and 10^7 CFU/mL) after 2 h of incubation for engineered phages and *E. coli*. (b) Peak current obtained from LSV curve of varying concentrations of *E. coli* after 1h (white bars), 2h (grey bars), and 3h (black bars) of incubation for phages and *E. coli*. One asterisk (*) represents a significant difference ($0.01 < p < 0.05$) and two asterisks (**) represents a significant difference ($p < 0.01$) between each treated group with the control group. Error bars represent standard deviation of six independent replicates.

increase of the binding time and levelled off after approximately 90 min. The binding time was therefore determined as 90 min. It was also noted that the signal increased more rapidly from 0 min to 30 min than that from 30 min to 90 min. This suggests that more enzymes bound on the gold surface at the first 30 min and then the binding rate became slower after 30 min.

Prior to the detection, NRGp7 phages were covalently conjugated with carboxylic acid groups functionalized MBs through the amide linkage. The use of magnetic separation facilitates the pre-concentration of target bacteria to improve the sensitivity of the detection. Additionally, it allows the separation of the target bacteria from food matrices, especially necessary for complex matrices which might interfere with the result.[35, 36] Bacteria separation using phage conjugated MBs is an improved alternative to immunomagnetic separation (IMS) which uses MBs coated with antibodies. Phages can offer many advantages over antibodies, such as specificity, robustness, and ability to distinguish viable cells. In addition, they are relatively stable in a large range of pH, temperature, and salt concentration, showing more potential to be applied in low resource settings.[37, 38] The effectiveness of phages as biorecognition elements for bacteria concentration has been investigated and reported in our previous studies with a capture efficiency of over 80% for 10^2 CFU/mL of *E. coli*.[22, 38] In this study, engineered phages immobilized on MBs captured the target *E. coli* as a biorecognition element and facilitated the detection by expressing reporter enzyme ALP for the subsequent detection.

The immobilization efficiency of phages on MBs was determined. An aliquot of 100 μL of MBs (2×10^9 beads/mL) after activation was mixed with 1 mL of (3.1 ± 0.3) $\times10^{10}$ PFU/mL NRGp7 phages. In the 1mL of mixture, the number of MBs in the tube was 2×10^8 (n), as specified by the manufacturer, and the original number of phages (N_0) was (3.1 ± 0.3) $\times10^{10}$ PFU. Following by the immobilization, the phages remaining in the supernatant (N_1) were determined as (0.9 ± 0.16) $\times$ 10^{10} PFU using a standard plaque assay. After subtracting the number of phages in the supernatant (N_1) from the original number of phages (N_0), the total number of phages conjugated onto the MBs ($N_2=N_0-N_1$) was determined to be (2.2 ± 0.4) $\times$ 10^{10} PFU. To estimate the number of phages on each particle, the total number of phages immobilized on MBs (N_2) was divided by the number of MBs used for the conjugation (n) following the equation N_2/n. Then the number of phages on each particle was therefore estimated to be 110 ± 4 PFU. The standard deviation was determined based on three independent trials. The capture of phage conjugated MBs was visualized using SEM. Fig. 4 shows the SEM images of *E. coli* cells, NRGp7 phages-MBs, and *E. coli* cells captured by the NRGp7 phages-MBs. These results demonstrated that NRGp7 phages were successfully conjugated to MBs and were able to be used to capture and infect the target *E. coli*.

Analytical performance of electrochemical detection of *E. coli* using NRGp7 phages

The analytical performance of this proposed electrochemical detection was carried out using varying concentrations of *E. coli* (10^4, 10^5, 10^6, and 10^7 CFU/mL). The detection of *E. coli* was based on measuring the activity of electrode-immobilized GBPs-ALP. The amount of GBPs-ALP expressed is related to the dynamic interaction of phage and *E. coli*, and the incubation time.[39] Therefore, we incubated samples after pre-concentration for 1, 2, and 3 h to determine an optimal time for the phage and *E. coli* incubation. T7 are lytic phages, so at the end of infection cycle the *E. coli* cells were lysed allowing the release of the reporter protein and replicated phages. Therefore, phage-based assays remove the need for the additional lysozyme or chemicals to open the cells for analysis.[37] The reporter enzyme ALP was then immobilized on gold electrode through the function of GBPs and quantified electrochemically. ALP catalyzed the hydrolysis of the substrate AAP to AA which facilitated the reduction of $AgNO_3$, leading to the deposition of Ag on the electrode surface that was quantified using linear sweep voltammetry. LSV curves were obtained for each concentration of *E. coli* and the peak current is proportional to the enzyme immobilized on the electrode. LSV

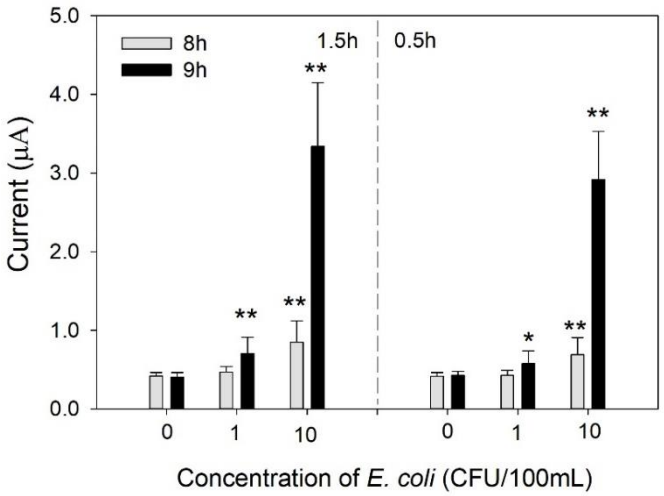

Figure 6: Results of electrochemical detection of 1 CFU and 10 CFU *E. coli* in 100 mL of drinking water after 8 h (grey bars) and 9h (black bars) of pre-incubation, respectively. The left figure used 1.5 h of for the binding of GBPs on the electrode and the right one was 0.5 h. One asterisk (*) represents a significant difference ($0.01 < p < 0.05$) and two asterisks (**) represents a significant difference ($p < 0.01$) between each treated group with the control group. Error bars represent standard deviation of ten replicates.

is a common electrochemical method used to measure the change of electron transfer in a relatively short response time. The peak current is proportional to the quantity of compound that is oxidized or reduced on the electrode.[40, 41] Using the measurement of the activity of ALP on the electrode, we were able to correlate the initial concentration of *E. coli*.

Fig. 5(a) shows electrochemical results after a 2 h incubation of phages and *E. coli*. These results suggested that the peak current of each variant increased with increasing *E. coli* concentration. The peak current was used as the electrochemical signal and the results from different incubation time were plotted Fig. 5(b). This figure shows that at each incubation time, the signal increased proportionally with the *E. coli* concentration. Similarly, with the increase in incubation time, the signal for each concentration of *E. coli* also increased due to the accumulation of enzyme expressed from engineered phages. The peak potential shifted slightly because the electrochemical system applied higher potentials to compensate the greater electrons diffusion when detecting high concentrations of *E. coli*. This potential shift did not significantly affect the performance of the method. From these results, an optimal incubation time for phage and *E. coli* was determined as 2 h. Because there was a significant increase on the signal from 1 to 2 h, while no significant increase from 2 to 3 h. Using this strategy, we were able to detect 10^5 CFU/mL after 2 h of incubation for phages and *E. coli*.

Electrochemical detection of *E. coli* in drinking water with pre-incubation

EPA regulations require that a suitable test for water quality has the ability to detect a single viable CFU of generic *E. coli* from 100 mL of water. Therefore, for the detection in real water samples, the ability to detect very low level of *E. coli* (1 CFU) is required. A pre-incubation step is normally used to allow for the growth of bacterial cells and the recovery of injured cells. Some chemical factors such as chlorine that used to treat the drinking water for disinfection may cause the injury to *E. coli* cells and affect the growth and various functions of *E. coli*. Thus, the drinking water sample was first treated with sodium thiosulfate to minimize the effect of chlorine on *E. coli* growth during the pre-incubation step. The assay was then performed following these steps: 1) separation and pre-concentration of target *E. coli* using phage-MBs, 2) incubation of phage-MBs captured *E. coli* for expression and release of enzyme, 3) immobilization of ALP on electrodes through the function of GBP, and 4) electrochemical detection. The drinking water samples inoculated with approximate 1 CFU, and 10 CFU of *E. coli* were pre-incubated for 8, 9, and 10 h, respectively. After pre-incubation, electrochemical detection results were obtained and shown in Fig. 6. Following 8 h of pre-incubation, we were able to detect as low as 10 CFU of *E. coli* in 100 mL drinking water and 1 CFU/100mL of *E. coli* after 9 h of pre-incubation. In terms of a rapid detection, the compromise between the assay time and detection limit should be considered. Because the binding efficiency of GBPs decreased after the initial 30 min of binding time, we reduced the binding time from 1.5 to 0.5 h. When the binding time of the enzyme was reduced to 0.5 h, a significant signal for 1 CFU/100mL of *E. coli* was still obtained after 9 h of pre-incubation. Therefore, the total assay time including pre-incubation, incubation of phage and *E. coli*, enzyme immobilization, and enzymatic reaction was 12 h to detect 1 CFU of *E. coli* in 100 mL of drinking water. There have been other rapid methods reported for the detection of *E. coli* in water samples based on the measurement of specific enzyme activity.[29, 42] Most of these studies utilized the intracellular enzyme of *E. coli*, our phage-based method provided a strategy to express other enzymes of interest used for different detection. Electrochemical methods show more potential to be miniaturized and used in low resource settings compared to fluorescent and colorimetric detection. Phages can also facilitate the electrochemical detection by expressing enzyme linked with a binding peptide, simplifying the procedure of electrode modification.

Conclusions

An electrochemical method based on engineered phages and gold binding peptides mediated enzyme immobilization was successfully developed for the detection of *E. coli* in drinking water. The engineered phages in this study were not only used to capture target bacteria as a biorecognition element, but also to facilitate the detection by producing the reporter protein. In addition, the expressed reporter protein has both enzyme activity and the ability to bind gold surface. This novel strategy using binding peptides to immobilize reporter enzymes on electrode is simple and take place directly without the need of additional chemicals or complicated procedure. We demonstrated the successful construction of NRGp7 phages, the expression of fusion protein GBPs-ALP from this phage, and the functionality of GBPs-ALP. The immobilization of proteins on solid surface using genetically fused specific binding peptides has the potential to extend to any solid surface with the use of

appropriate binding peptides and for the application in a wide range of biosensor development. Engineered phages were also conjugated with MBs to separate and pre-concentrate the target bacteria in order to improve the sensitivity. The EPA method 1603 which utilizes membrane filtration, is one of the most widely used method for the enumeration of *E. coli* in drinking water. This assay is simple to perform and inexpensive, however it requires at least 24 hours of incubation period. In our study, 10^5 CFU/mL *E. coli* was detected within 4 hours. After 9 hours of pre-incubation, 1 CFU of *E. coli* in 100 mL of drinking water was able to be detected with a total assay time of 12 hours. These experiments demonstrate a proof-of-principle assay format which can be expanded using other phages or phage cocktails for specific host ranges. While T7 has demonstrated specificity within *E. coli* (supporting information), a more comprehensive coverage of the required host range is typically accomplished using phage cocktails. Given the significant benefits that phage-based tools have demonstrated as both detection and therapy tools, many researcher have now shifted focus to the engineering of phage specificity using genetic engineering of tail fibers.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

References

1. United Nations, 64/292. The human right to water and sanitation, (accessed July, 2010).
2. T. Brick, B. Primrose, R. Chandrasekhar, S. Roy, J. Muliyil and G. Kang, *International journal of hygiene and environmental health*, 2004, **207**, 473-480.
3. D. D. Marino, *Journal of the American Dietetic Association*, 2007, **107**, 1930-1934.
4. S. C. Edberg, E. W. Rice, R. J. Karlin and M. J. Allen, *Symp Ser Soc Appl Microbiol*, 2000, 106S-116S.
5. L. Fewtrell and J. Bartram, *Water Quality: Guidelines, Standards & Health*, IWA publishing, 2001.
6. EPA, National Primary Drinking Water Regulations (NPDWRs), https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations, (accessed May, 2009).
7. EPA, Analytical Methods Approved for Compliance Monitoring under the Ground Water Rule, https://www.epa.gov/sites/production/files/2017-02/documents/gwr_approved_methods.pdf, (accessed Feburary, 2017).
8. D. Ivnitski, I. Abdel-Hamid, P. Atanasov, E. Wilkins and S. Stricker, *Electroanalysis*, 2000, **12**, 317-325.
9. D. Wang, J. Chen and S. R. Nugen, *Analytical chemistry*, 2017, **89**, 1650-1657.
10. M. Xu, R. Wang and Y. Li, *Talanta*, 2017, **162**, 511-522.
11. D. W. Kimmel, G. LeBlanc, M. E. Meschievitz and D. E. Cliffel, *Analytical chemistry*, 2011, **84**, 685-707.
12. H. Yang, *Current opinion in chemical biology*, 2012, **16**, 422-428.
13. F. Rusmini, Z. Zhong and J. Feijen, *Biomacromolecules*, 2007, **8**, 1775-1789.
14. M. T. Neves‐Petersen, T. Snabe, S. Klitgaard, M. Duroux and S. B. Petersen, *Protein Science*, 2006, **15**, 343-351.
15. M. Sarikaya, C. Tamerler, A. K.-Y. Jen, K. Schulten and F. Baneyx, *Nature materials*, 2003, **2**, 577.
16. J. Yang, S.-E. Kim, M. Cho, I.-K. Yoo, W.-S. Choe and Y. Lee, *Biosensors and Bioelectronics*, 2014, **61**, 38-44.
17. T. Kacar, M. T. Zin, C. So, B. Wilson, H. Ma, N. Gul‐Karaguler, A. K. Y. Jen, M. Sarikaya and C. Tamerler, *Biotechnology and bioengineering*, 2009, **103**, 696-705.
18. T. J. Park, S. Y. Lee, S. J. Lee, J. P. Park, K. S. Yang, K.-B. Lee, S. Ko, J. B. Park, T. Kim and S. K. Kim, *Analytical chemistry*, 2006, **78**, 7197-7205.
19. D. Rakhuba, E. Kolomiets, E. S. Dey and G. Novik, *Pol. J. Microbiol*, 2010, **59**, 145-155.
20. A. Singh, D. Arutyunov, C. M. Szymanski and S. Evoy, *Analyst*, 2012, **137**, 3405-3421.
21. E. Fernandes, V. Martins, C. Nóbrega, C. Carvalho, F. Cardoso, S. Cardoso, J. Dias, D. Deng, L. Kluskens and P. Freitas, *Biosensors and Bioelectronics*, 2014, **52**, 239-246.
22. J. Chen, B. Duncan, Z. Wang, L.-S. Wang, V. M. Rotello and S. R. Nugen, *Nanoscale*, 2015, **7**, 16230-16236.
23. R. Derda, M. R. Lockett, S. K. Tang, R. C. Fuller, E. J. Maxwell, B. Breiten, C. A. Cuddemi, A. Ozdogan and G. M. Whitesides, *Analytical chemistry*, 2013, **85**, 7213-7220.
24. G. F. Hatfull and R. W. Hendrix, *Current Opinion in Virology*, 2011, **1**, 298-303.
25. D. P. Pires, S. Cleto, S. Sillankorva, J. Azeredo and T. K. Lu, *Microbiology and Molecular Biology Reviews*, 2016, **80**, 523-543.
26. J. McCafferty, R. H. Jackson and D. J. Chiswell, *Protein Engineering, Design and Selection*, 1991, **4**, 955-961.
27. S. Alcaine, D. Pacitto, D. Sela and S. Nugen, *Analyst*, 2015, **140**, 7629-7636.
28. A. A. Jackson, T. C. Hinkley, J. N. Talbert, S. R. Nugen and D. A. Sela, *Analyst*, 2016, **141**, 5543-5548.
29. J. Chen, S. D. Alcaine, Z. Jiang, V. M. Rotello and S. R. Nugen, *Analytical chemistry*, 2015, **87**, 8977-8984.
30. S. Alcaine, L. Tilton, M. Serrano, M. Wang, R. Vachet and S. Nugen, *Applied microbiology and biotechnology*, 2015, **99**, 8177-8185.

31. T. C. Hinkley, S. Singh, S. Garing, A.-L. M. Le Ny, K. P. Nichols, J. E. Peters, J. N. Talbert and S. R. Nugen, *Scientific Reports*, 2018, **8**, 14630.
32. A. Rompré, P. Servais, J. Baudart, M.-R. de-Roubin and P. Laurent, *Journal of Microbiological Methods*, 2002, **49**, 31-54.
33. V. L. Sirisha, A. Jain and A. Jain, in *Advances in Food and Nutrition Research*, eds. S.-K. Kim and F. Toldrá, Academic Press, 2016, vol. 79, pp. 179-211.
34. S. Brown, *Nature biotechnology*, 1997, **15**, 269-272.
35. D. Wright, P. Chapman and C. Siddons, *Epidemiology & Infection*, 1994, **113**, 31-39.
36. D. Wang, Z. Wang, J. Chen, A. J. Kinchla and S. R. Nugen, *Food Control*, 2016, **62**, 81-88.
37. R. Peltomaa, I. Lopez-Perolio, E. Benito-Pena, R. Barderas and M. C. Moreno-Bondi, *Analytical and Bioanalytical Chemistry*, 2016, **408**, 1805-1828.
38. Z. Wang, D. Wang, A. J. Kinchla, D. A. Sela and S. R. Nugen, *Analytical and bioanalytical chemistry*, 2016, 1-10.
39. S. D. Alcaine, K. Law, S. Ho, A. J. Kinchla, D. A. Sela and S. R. Nugen, *Biosensors and Bioelectronics*, 2016, **82**, 14-19.
40. B. Qu, L. Guo, X. Chu, D.-H. Wu, G.-L. Shen and R.-Q. Yu, *Analytica chimica acta*, 2010, **663**, 147-152.
41. G. Lai, F. Yan, J. Wu, C. Leng and H. Ju, *Analytical chemistry*, 2011, **83**, 2726-2732.
42. N. Hesari, A. Alum, M. Elzein and M. Abbaszadegan, *Enzyme and Microbial Technology*, 2016, **83**, 22-28.