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Washing- and Separation-Free Electrochemical Detection of *Porphyromonas gingivalis* in Saliva for Initial Diagnosis of Periodontitis

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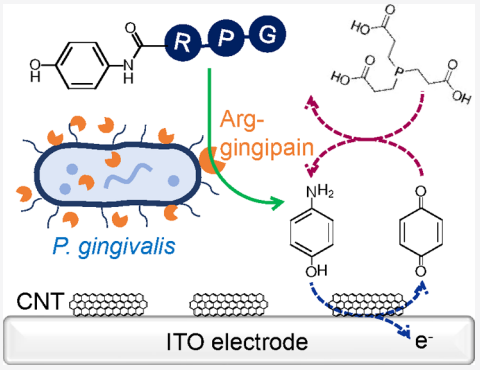
ABSTRACT: Indirect detection of *Porphyromonas gingivalis* in saliva, based on proteolytic cleavage by an Arg-specific gingipain (Arg-gingipain), has traditionally been used for simple, initial diagnosis of periodontitis. To accurately detect *P. gingivalis* using a point-of-care format, development of a simple biosensor that can measure the exact concentration of *P. gingivalis* is required. However, electrochemical detection in saliva is challenging due to the presence of various interfering electroactive species in different concentrations. Here, we report a washing- and separation-free electrochemical biosensor for sensitive detection of *P. gingivalis* in saliva. Glycine−proline−arginine conjugated with 4-aminophenol (AP) was used as an electrochemical substrate for a trypsin-like Arg-gingipain, and glycylglycine was used to increase the Arg-gingipain activity. The electrochemical signal of AP was increased using electrochemical−chemical (EC) redox cycling involving an electrode, AP, and tris(2-carboxyethyl)phosphine, and the electrochemical charge signal was corrected using the initial charge obtained before a 15 min incubation period. The EC redox cycling combined with the matrix-corrected signal facilitated a high and reproducible signal without requiring washing and separation steps. The proteolytic cleavage of the electrochemical substrate was specific to *P. gingivalis*. The calculated detection limit for *P. gingivalis* in artificial saliva was 5×10^5 colony-forming units/mL, and the concentration of *P. gingivalis* in human saliva could be measured. The developed biosensor can be used as an initial diagnosis method to distinguish between healthy people and patients with periodontal diseases.



Bacterial proteases play an important role in bacterial proliferation in host cells and can cause serious toxicity.[1] Such proteases can be used as specific biomarkers for detecting protease-secreting pathogenic bacteria.[2,3] Gingipains (Arg-specific and Lys-specific), which are trypsin-like proteases secreted by *Porphyromonas gingivalis* (*P. gingivalis*), destroy periodontal connective tissues and the blood clotting system, leading to periodontal inflammation and bleeding, respectively, and resulting in alveolar bone loss.[4] Among the two, the Arg-specific gingipain (Arg-gingipain) plays a major role in the virulence of *P. gingivalis*.[5] Therefore, the indirect detection of *P. gingivalis* using Arg-gingipain in saliva has been employed for a simple, initial diagnosis of periodontitis.[2,6,7]

The N_α-benzoyl-DL-arginine-β-naphthylamide (BANA) test is a commercial point-of-care method for indirect detection of *P. gingivalis*.[8−12] Arg-gingipain cleaves the peptide bond between arginine and β-naphthylamide of BANA, and the released β-naphthylamide produces a colorimetric signal with a maximum wavelength of 410 nm. The BANA test has also been used to detect other proteases such as trypsin,[13,14] cathepsin B1,[15] cathepsin H,[16] and papain.[15,17] However, the BANA test divides the concentration of *P. gingivalis* into positive, weakly positive, and negative only depending on the color intensity of the test strip, which is visually identified. To accurately detect *P. gingivalis* in saliva with a point-of-care format, development of a biosensor that can measure the exact concentration of *P. gingivalis* is required.

Direct detection of *P. gingivalis* using methods such as (i) culture-based detection,[11,18,19] (ii) DNA detection using polymerase chain reaction (PCR) and loop-mediated isothermal amplification,[11,18,20] and (iii) affinity binding-based detection (i.e., immunoassay)[21] has been commonly used to measure the exact concentration of *P. gingivalis*. Although these methods can detect *P. gingivalis* with better sensitivity, specificity, and accuracy than the BANA test, the detection procedures are complex and time-consuming, which are unsuitable for straightforward point-of-care detection.

Electrochemical detection has been a prime candidate for point-of-care detection, as it allows for quantitative measure-

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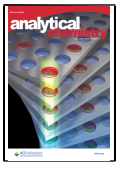

ment along with easy miniaturization.[22] Indeed, many electrochemical methods have been developed for non-invasive detection of salivary biomarkers to diagnose various diseases, including bacterial or viral infections.[23] Electrochemical methods based on proteolytic cleavage have also been developed for straightforward point-of-care detection.[24,25] However, electrochemical detection of *P. gingivalis* (or gingipain) based on proteolytic cleavage has not yet been attempted.

The absence of washing and separation steps is an essential requirement for straightforward point-of-care detection. However, it is difficult to obtain low and reproducible electrochemical background levels without washing and separation because saliva samples have different concentrations of various electroactive species.[6,23] To achieve a high signal-to-background ratio without such steps, an electrochemical signal value obtained in the presence of a target should be high and/or the signal level should be corrected by the value obtained in the absence of the target. The electrochemical signal of an electroactive species released by proteolytic cleavage can be substantially increased by using redox cycling schemes such as electrochemical−chemical (EC) redox cycling and electrochemical−enzymatic redox cycling.[24,26] Because the electrochemical signal correction is more effective when the electrochemical signal level is high, it is expected that a good signal-to-background ratio can be obtained when the electrochemical signal correction is combined with a redox cycling scheme. However, to date, such combined use has not been reported. In many cases, two solutions or two working electrodes are used for the electrochemical signal correction.[27] It is required to use a single salivary sample and a single working electrode for straightforward point-of-care detection.

Herein, we demonstrate a washing- and separation-free electrochemical biosensor for indirect detection of *P. gingivalis* based on proteolytic cleavage by Arg-gingipain (Figure 1). An

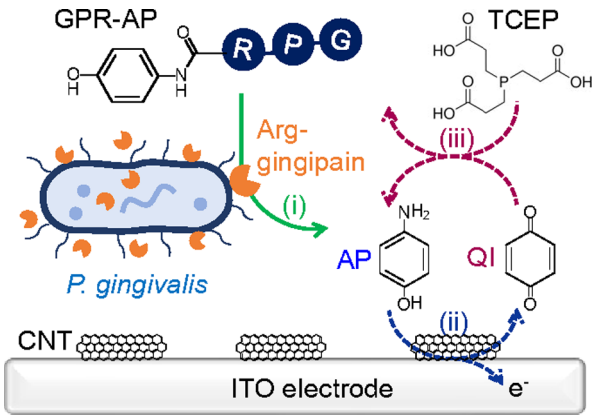

Figure 1. Schematic diagram of electrochemical detection of *P. gingivalis* based on proteolytic cleavage of an electrochemical substrate by Arg-gingipain.

electrochemical trypsin substrate containing 4-aminophenol (AP) was used for the proteolytic cleavage, which released AP from the substrate. EC redox cycling involving AP was combined with matrix correction to obtain a high signal-to-background ratio. Specific detection of *P. gingivalis* was examined using various bacteria that do not secrete Arg-gingipain. The developed electrochemical biosensor was applied to the detection of *P. gingivalis* in artificial saliva.

EXPERIMENTAL SECTION

Chemicals and Solutions. Two electrochemical substrates of trypsin-like *P. gingivalis* [glycine−proline−arginine conjugated with AP (GPR-AP) and N_α-benzoyl-DL-arginine conjugated with AP (benzoyl-R-AP)] were obtained from Anygen Co. Ltd. (Jangseong-gun, Korea). AP, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), carboxylated multi-walled carbon nanotube (CNT), glycylglycine (Gly−Gly), cysteine, BANA, bovine serum albumin (BSA), and all reagents used for the preparation of buffer solutions were obtained from Sigma-Aldrich. Artificial saliva was purchased from Pickering Laboratories (Mountain View, CA, USA). Indium tin oxide (ITO) electrodes were purchased from Corning Co. (Daegu, Korea).

Preparation of *P. gingivalis* and Other Bacteria. The bacterial strains used in this study are listed in Table S1 of the Supporting Information. *P. gingivalis*, *P. gingivalis* MT10, *P. gingivalis* MT10w, *Treponema denticola*, *T. forsythia*, *Porphyromonas intermedia*, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum* were grown in a Gifu anaerobic medium (Nissui Pharmaceutical, Japan) broth at 37 °C in an anaerobic chamber (anaerobic system model 1025, Forma scientific, USA) containing 90% N_2, 5% H_2, and 5% CO_2. *Aggregatibacter actinomycetemcomitans* was grown in a tryptic soy broth (Becton & Dickinson, USA) medium supplemented with 1% yeast extract at 37 °C in a 5% CO_2 incubator. *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Neisseria mucosa* were grown in a brain heart infusion (BHI, Difco, USA) broth at 37 °C in a 5% CO_2 incubator. *Escherichia coli* and *Escherichia faecalis* were grown in a BHI broth under aerobic conditions at 37 °C. After 72 h of culture, the bacteria were pelleted by centrifugation for 10 min at 10,000*g*. Crude samples were used immediately or stored at −20 °C for later use. Media containing 10^9 colony-forming units (CFU)/mL *P. gingivalis* and other bacteria were filtered by centrifugation through a filter (cut-off molecular weight = 30,000 Da) for 20 min at 13,500*g* to remove the medium before electrochemical measurement.

Saliva Collection. Whole saliva was obtained from all study subjects using Salivette (Sarstedt, Numbrecht, Germany). The experimental protocol was approved by the Institutional Review Board of Pusan National University (PNUDH-2017-023). Written informed consent was obtained from all participants before the study. All subjects were asked to rinse their mouth with distilled water 5 min prior to saliva collection. At the time of saliva collection, each subject placed a cotton ball in their mouth for 5 min. The cotton ball was subsequently removed and transferred to a plastic tube. The collected samples were centrifuged and the saliva samples were stored at −80 °C until further analysis.

Electrode Preparation and Electrochemical Measurement. ITO electrodes (1 cm × 2 cm) were pretreated by dipping them in a mixed solution of H_2O, H_2O_2 (30%) and NH_4OH (30%) at a volume ratio of 5:1:1 for 1 h at 70 °C.[28] To prepare CNT-modified ITO electrodes, pretreated ITO electrodes were immersed for 3 s at 25 °C in distilled water containing 0.2 mg/mL of dispersed multi-walled CNTs. Afterward, 70 μL of phosphate-buffered saline (pH 7.4) containing 1% (w/v) BSA was dropped onto the CNT-modified ITO electrodes and maintained at 4 °C for 30 min. The CNT- and BSA-modified ITO electrodes were rinsed by distilled water and dried by nitrogen gas.

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A Teflon electrochemical cell was assembled with a CNT- and BSA-modified ITO electrode, an Ag/AgCl reference electrode (3 M NaCl), and a platinum counter electrode. The exposed geometric area of the working electrode was approximately 0.28 cm^2. Electrochemical measurements were performed using a CHI 1040C potentiostat (CH Instruments, Austin, TX). To detect *P. gingivalis* in saliva, 300 μL of artificial saliva or clinical saliva containing various concentrations of *P. gingivalis* was mixed with 200 μL of 50 mM Tris buffer (pH 7.5) containing 0.25 mM GPR-AP, 5 mM TCEP, and 125 mM Gly−Gly. The mixed solution was injected into an electrochemical cell. The final concentrations of GPR-AP, TCEP, and Gly−Gly were 0.1, 2, and 50 mM, respectively. Electrochemical measurements were performed before and after incubating the electrochemical cells for 15 min at 55 °C.

RESULTS AND DISCUSSION

In general, electrochemical detection of proteases is based on (i) the release of an electroactive species from an electrochemical peptide substrate by proteolytic cleavage (signal-on scheme)[24,29] or (ii) the detachment of an electroactive label from an electrode by proteolytic cleavage (signal-off scheme).[30] Most trypsin detection methods involve these two schemes. The signal-on scheme is favorable compared to the signal-off scheme at obtaining a low detection limit.[25] Therefore, the signal-on scheme was used for the detection of a trypsin-like Arg-gingipain. Several peptides conjugated with a proteolytically cleaved electroactive species have been known as electrochemical substrates for trypsin detection.[29] Because GPR-AP is the most common electrochemical substrate, it was used for Arg-gingipain detection based on the signal-on scheme. Arg-gingipain cleaves the peptide bond mainly at the carboxyl side of lysine or arginine (R) in a peptide.[2,31] In comparison, benzoyl-R-AP was also examined as an electrochemical substrate corresponding to the colorimetric substrate BANA.[8−12]

If AP is released from GPR-AP and benzoyl-R-AP by Arg-gingipain, AP can be electrochemically oxidized to obtain an electrochemical signal. To increase the anodic current of AP, EC redox cycling involving an electrode, AP, and TCEP[29] was used. Because highly electrocatalytic electrodes, such as the Au electrode, showed rapid direct oxidation of TCEP, low electrocatalytic ITO electrodes modified partially with electrocatalytic CNTs[32] were used to obtain a high electrochemical signal-to-background ratio. The preparation of the modified ITO electrode was optimized according to the method shown in Figure S1. The ITO electrode immersed for 3 s in water containing carboxylated multi-walled CNTs showed the highest electrochemical signal-to-background ratio. In Figure 2a, it is shown that the anodic current for AP substantially increased in the presence of TCEP, indicating that EC redox cycling facilitated the production of a higher electrochemical signal.

Proteolytic cleavage of GPR-AP by Arg-gingipain in *P. gingivalis* was first investigated using cyclic voltammograms (Figure 2b). The anodic current slightly increased in the presence of *P. gingivalis* by approximately 0.3 V (curves i and iii). It is known that Gly−Gly and cysteine act as activators of proteolytic cleavage by Arg-gingipain.[7] Indeed, the anodic current substantially increased in the presence of Gly−Gly and *P. gingivalis* (curves ii, iii and iv of Figure 2b). In the chronocoulograms, an increase of the anodic charge was clearly observed in the presence of Gly−Gly (Figure 2c). The same

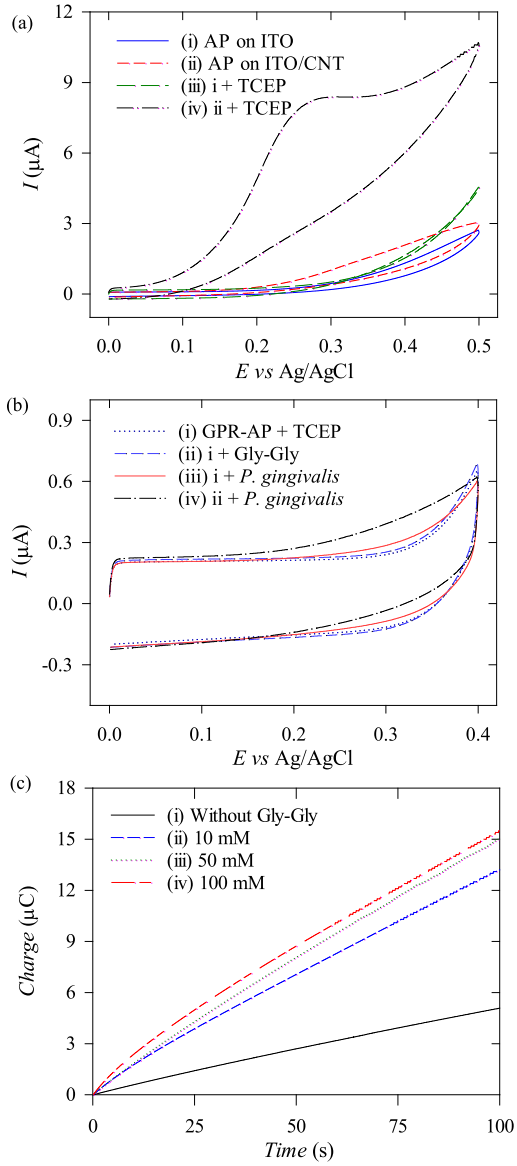

Figure 2. Cyclic voltammograms (at a scan rate of 20 mV/s) obtained at CNT- and BSA-modified ITO electrodes (a) in Tris buffer (pH 7.5) containing (i) 0.1 mM AP and (ii) 0.1 mM AP and 2.0 mM TCEP and (b) after an incubation period of 15 min at 55 °C in Tris buffer (pH 7.5) containing (i) 0.1 mM GPR-AP and 2.0 mM TCEP; (ii) 0.1 mM GPR-AP, 2.0 mM TCEP, and 50 mM Gly−Gly; (iii) 0.1 mM GPR-AP, 2.0 mM TCEP, and 10^7 CFU/mL *P. gingivalis*; and (iv) 0.1 mM GPR-AP, 2.0 mM TCEP, 10^7 CFU/mL *P. gingivalis*, and 50 mM Gly−Gly. (c) Chronocoulograms obtained (at 0.25 V) at CNT- and BSA-modified ITO electrodes after an incubation period of 15 min at 55 °C in Tris buffer (pH 7.5) containing 0.1 mM GPR-AP, 2.0 mM TCEP, 10^7 CFU/mL, and (i) 0, (ii) 10, (iii) 50, or (iv) 100 mM Gly−Gly.

increasing behavior was also observed in the presence of cysteine (Figure S2). However, the electrochemical background charge substantially increased in the presence of cysteine (Figure S2), although it did not increase in the presence of Gly−Gly. The signal increases for 10, 50, and 100 mM Gly−Gly were comparable (Figure 2c), and 50 mM Gly−Gly was used for further experiment. When benzoyl-R-AP was examined as an electrochemical substrate, it was not cleaved by Arg-gingipain, although GPR-AP was cleaved (Figure S3). Therefore, benzoyl-R-AP could not be used. The applied

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potential, the solution pH, and the incubation temperature were optimized to obtain the highest signal-to-background ratio (Figure 3). The background and signal levels were increased by increasing the applied potential, and the highest signal-to-background ratio was obtained at 0.3 V (Figure 3a). However, 0.25 V was chosen because the electrochemical background in human saliva became high at 0.3 V. The signal-to-background ratios obtained in five solutions (pH 5.5, 6.5, 7.5, 8.5, and 9.5) are shown in Figure 3b. The highest signal level was obtained at pH 7.5 because the activity of Arg-gingipain is high in neutral pHs. The optimal reaction temperature of Arg-gingipain is known as 55 °C.[10,33,34] The background levels obtained at three temperatures (35, 45, and 55 °C) were similar, while the signal level was increased when the reaction temperature increased (Figure 3c).

To investigate the selectivity of the developed biosensor, chronocoulometric charges (Figure 4) and absorbance data

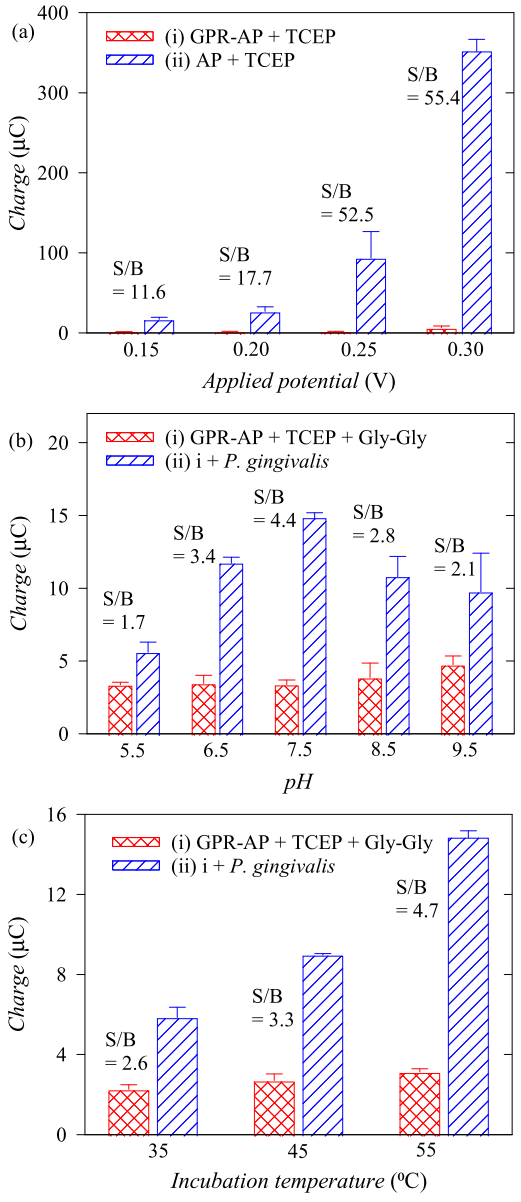

Figure 3. Histograms for optimal conditions of *P. gingivalis* detection. Charge values measured at 100 s in the chronocoulograms obtained at CNT- and BSA-modified ITO electrodes (a) at four applied potentials (0.15, 0.20, 0.25, and 0.30 V) in Tris buffer (pH 7.5) containing (i) 0.1 mM GPR-AP and 2.0 mM TCEP and (ii) 0.1 mM AP and 2.0 mM TCEP; (b) at 0.25 V after an incubation period of 15 min at 55 °C in five pH solutions of Tris buffer (pH 5.5, 6.5, 7.5, 8.5, and 9.5) containing (i) 0.1 mM GPR-AP, 2.0 mM TCEP, and 50 mM Gly−Gly and (ii) 0.1 mM GPR-AP, 2.0 mM TCEP, 50 mM Gly−Gly, and 10^7 CFU/mL *P. gingivalis*; and (c) at 0.25 V after an incubation period of 15 min at three temperature (35, 45, and 55 °C) in Tris buffer (pH 7.5) containing (i) 0.1 mM GPR-AP, 2.0 mM TCEP, and 50 mM Gly−Gly and (ii) 0.1 mM GPR-AP, 2.0 mM TCEP, 50 mM Gly−Gly, and 10^7 CFU/mL *P. gingivalis*.

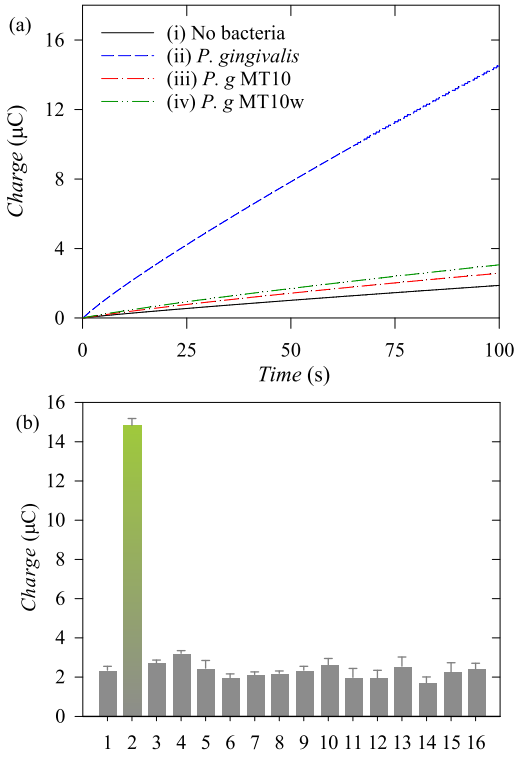

Figure 4. (a) Chronocoulograms obtained (at 0.25 V) at CNT- and BSA-modified ITO electrodes after an incubation period of 15 min at 55 °C in a mixed solution of Tris buffer (pH 7.5) containing 0.1 mM GPR-AP, 2.0 mM TCEP, and 50 mM Gly−Gly and artificial saliva containing (i) no bacteria, 10^7 CFU/mL (ii) *P. gingivalis*, (iii) *P. gingivalis* MT10, and (iv) *P. gingivalis* MT10w. (b) Comparison of the charge values measured at 100 s in the chronocoulograms in panel (a). Artificial saliva contained 10^7 CFU/mL bacteria [1: no bacteria, 2: *P. gingivalis*, 3: *P. gingivalis* MT10, 4: *P. gingivalis* MT10w, 5: *T. denticola*, 6: *T. forsythia*, 7: *S. mitis*, 8: *S. salivarius*, 9: *P. intermedia*, 10: *A. actinomycetemcomitans*, 11: *P. endodontalis*, 12: *F. nucleatum*, 13: *E. coli*, 14: *E. faecalis*, 15: *N. mucosa*, and 16: *S. mutans*].

(Figure S4) were obtained using 15 different bacteria [*P. gingivalis*, *P. gingivalis* MT10, *P. gingivalis* MT10w, *T. denticola*, *T. forsythia*, *S. mitis*, *S. salivarius*, *P. intermedia*, *A. actinomycetemcomitans*, *P. endodontalis*, *F. nucleatum*, *E. coli*, *E. faecalis*, *N. mucosa*, and *S. mutans*]. In Figure 4a, it is shown that the charge slope increased in the presence of *P. gingivalis*, which can cleave the peptide bond at the carboxyl side of both lysine and arginine.[31] However, there was no considerable change in the slope in the presence of *P. gingivalis* MT10[35] that can cleave the peptide bond at the carboxyl side of only lysine. The same behavior was also observed with *P. gingivalis* MT10w,[35] which cannot cleave the peptide bond at the carboxyl side of lysine and arginine. These results clearly show that the specific cleavage of the peptide bond next to the

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arginine residue of GPR-AP occurred only in the presence of *P. gingivalis*. Figure 4b shows that, of all the bacteria studied, only *P. gingivalis* could release AP from GPR-AP via the specific cleavage. The same trend was also found in the absorbance measurement at 380 nm obtained using BANA (Figure S4). All results confirmed that the proteolytic cleavage of GPR-AP is specific to *P. gingivalis*. The chronocoulometric charge data (Figure 4b) showed more clear distinction for *P. gingivalis* than the BANA-based absorbance data (Figure S4).

Artificial salivary solutions spiked with various concentrations of *P. gingivalis* were used to examine the feasibility of *P. gingivalis* detection using this proteolytic cleavage to investigate whether chronocoulometric data depend on the concentration of *P. gingivalis* (Figure 5). As the concentration of *P. gingivalis* increased, the charge slope obtained after the 15 min incubation increased (Figure 5a). However, the initial charge slope for the matrix obtained before the 15 min incubation increased as the concentration of *P. gingivalis* increased (Figure S5), although the amount of released AP was negligible before the incubation. This result indicated that a matrix effect was considerable. This matrix effect was more severe in human saliva because different concentrations of various electroactive species are present in saliva.[23]

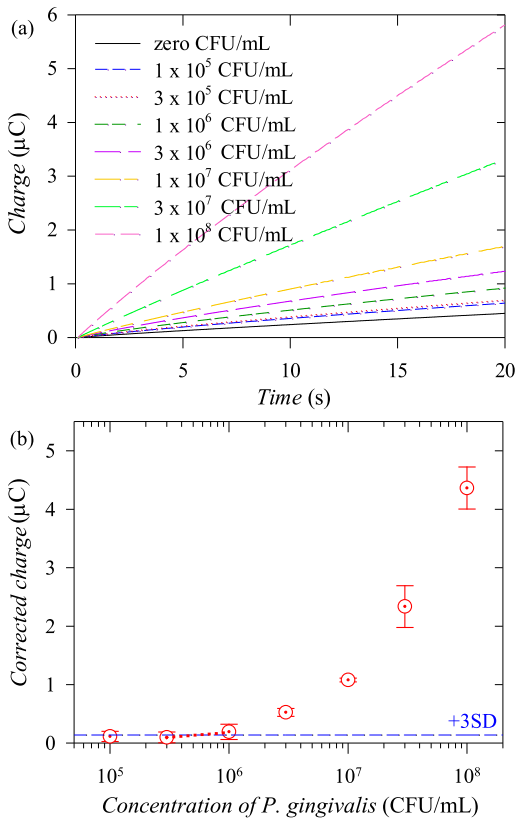

Figure 5. (a) Chronocoulograms obtained (at 0.25 V) using the scheme of Figure 1 in artificial saliva containing various concentrations of *P. gingivalis* at CNT- and BSA-modified ITO electrodes after an incubation period of 15 min at 55 °C in Tris buffer (pH 7.5) containing 0.1 mM GPR-AP, 2.0 mM TCEP, and 50 mM Gly−Gly. (b) Calibration plot obtained matrix-corrected charges. All experiments were conducted using three different electrodes for each sample. The error bars represent 1 SD of the measured values. All data were subtracted from the mean value determined from seven measurements at zero concentration. The dashed line corresponds to +3SD of the charge value at zero concentration.

To accurately measure the concentration of *P. gingivalis*, the correction of electrochemical data was required. In many cases, two solutions or two working electrodes are used for correction of electrochemical signals.[27] To use a single salivary sample and a single working electrode, the matrix-corrected charges were calculated by subtracting the initial charge at 20 s in the chronocoulogram obtained before the 15 min incubation (Figure S5) from the signal charge at 20 s in the chronocoulogram obtained after the incubation (Figure 5a). The calibration plot obtained using the matrix-corrected charges is shown in Figure 5b. The detection limit corresponds to the concentration at which the mean is equal to 3 times the standard deviation (SD) (+3SD in Figure 5b) at a concentration of zero. The calculated detection limit for *P. gingivalis* in artificial saliva was 5×10^5 CFU/mL, which satisfies the required detection limit for the initial diagnosis of periodontitis. *P. gingivalis* in the medium was pretreated by centrifugation using a filter (cut-off molecular weight = 30,000 Da) to remove the culture medium before spiking in artificial saliva. Since Arg-gingipain (molecular weight = ~50,000 Da) was partially lost in the pretreatment process,[36] the actual detection limit for *P. gingivalis* was expected to be lower. The matrix correction, as well as EC redox cycling, facilitated sensitive detection. Table S2 shows comparison of the developed method with other methods. The traditional BANA test only reports non-quantitative results such as negative, weakly positive, and positive results.[8−11] Although the detection using PCR and affinity binding shows lower or similar detection limits,[20,21] it requires a long detection time and complicated detection processes. However, the developed method allowed quantitative and simple detection with a short detection time.

Before applying the developed biosensor to the detection of *P. gingivalis* in human saliva, another feasibility experiment was performed. Two chronocoulograms for two same human saliva samples (one spiked with no *P. gingivalis* and another spiked with 10^7 CFU/mL *P. gingivalis*) were obtained before and after the 15 min incubation steps (Figure S6). In both samples, the charge slope obtained after the incubation was higher than that obtained before the incubation. The difference between the two slopes obtained after and before the incubation for the spiked sample was substantially larger than the difference for the unspiked sample, although the two slopes obtained before the incubation for the two samples were different. This result indicates that the matrix-corrected signal increased with the increasing concentration of *P. gingivalis* in human saliva.

Finally, the concentrations of *P. gingivalis* for 30 saliva samples collected from healthy subjects and 30 saliva samples collected from periodontitis patients were measured using the developed biosensor (Figure 6). Using our developed sensor, the measured concentration of *P. gingivalis* was 6.52 ± 0.49 log CFU/mL and 7.15 ± 0.47 log CFU/mL in the healthy human saliva sample and the periodontal disease saliva sample, respectively (p value = 0.000007). In most previous studies, the presence of *P. gingivalis* is determined by real-time PCR methods, and quantification of the bacteria is limited. *P. gingivalis* was detected in around 25% of healthy subjects, while it was detected in around 80% of periodontitis patients.[37] When the minimum positive threshold was set at 6.8 log CFU/ml, the positive rate was 20% in healthy subjects and 77% in periodontitis patients. Thus, the developed sensor can be used

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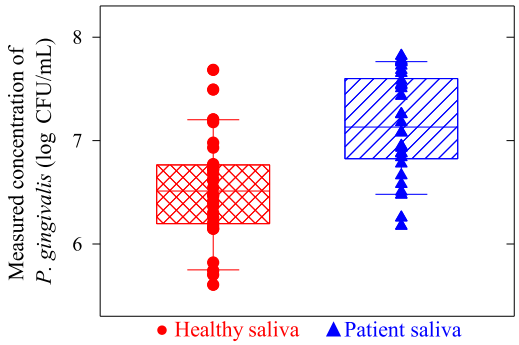

Figure 6. Comparison of the concentrations of *P. gingivalis* measured using the developed biosensor for 30 clinically healthy saliva samples and 30 periodontitis saliva samples.

not only to quantify *P. gingivalis* concentration in saliva but also to distinguish between healthy people and periodontitis patients with optimal threshold.

CONCLUSIONS

We have shown that electrochemical detection of *P. gingivalis* in human saliva containing different concentrations of various interfering electroactive species can be performed without the inclusion of washing or separation steps. GPR-AP was used as an electrochemical substrate of trypsin-like Arg-gingipain, and Gly−Gly was used to increase the Arg-gingipain activity. The EC redox cycling combined with the matrix-corrected signal facilitated high and reproducible signal levels without washing and separation steps. The electrochemical signal of AP was increased by EC redox cycling involving a CNT- and BSA-modified ITO electrode, AP, and TCEP, and the matrix-corrected signal was obtained using a single working electrode and a single saliva sample. The proteolytic cleavage of GPR-AP was highly specific to *P. gingivalis*. The chronocoulometric charge data showed more clear distinction for *P. gingivalis* than the BANA-based absorbance data. The calculated detection limit for *P. gingivalis* in artificial saliva was 5×10^5 CFU/mL. The developed biosensor is promising for straightforward point-of-care testing that allows for initial diagnosis of periodontitis. Moreover, it is expected that this detection method can be applied to detect microorganisms containing secreting or membrane-embedded proteases that cleave specific amino acid sequences [e.g., *E. coli* containing outer membrane protease (OmpT)].

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c00572.

Histograms obtained under different conditions of electrode preparation; chronocoulograms obtained under different conditions; and selectivity test using absorbance measurement (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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