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Amplified QCM biosensor for type IV collagenase based on collagenase-cleavage of gold nanoparticles functionalized peptide

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ABSTRACT

The present study develops a rapid, simple and efficient method for the determination of type IV collagenase by using a specific peptide-modified quartz crystal microbalance (QCM). A small peptide (P1), contains a specific sequence (Pro-Gly) and a terminal cysteine, was synthetized and immobilized to the surface of QCM electrode via the reaction between Au and thiol of the cysteine. The peptide bond between proline and glycine can be specific hydrolyzed cleavage by type IV collagenase, which enabled the modified electrode with a high selectivity toward type IV collagenase. The cleaving process caused a frequency change of QCM to give a signal related to the concentration of type IV collagenase. The morphologies of the modified electrodes were characterized by scanning electron microscope (SEM) and the specific hydrolyzed cleavage process was monitored by QCM. When P1 was modified with gold nanoparticles (P1-Au NPs), the signal could be amplified to further enhance the sensitivity of the designed sensor due to the high-mass of the modified Au NPs. Compared the direct unamplified assay, the values obtained for the limit of detection for type IV collagenase was 0.96 ng mL^{-1}, yielding about 6.5 times of magnitude improvement in sensitivity. This signal enhanced peptide based QCM biosensor for type IV collagenase also showed good selectivity and sensitivity in complex matrix.

1. Introduction

As a family of zinc-dependent neutral metalloproteinases, collagenases are important proteolytic tools for extracellular matrix remodeling during organ development and tissue regeneration, but they also apparently play important roles in many pathological situations (Olczyk et al., 2014; Sano et al., 2004). For example, Type IV collagenase can degrade type IV collagen, which is the component of cell membranes in various tissues, such as skin, heart, blood vessels, cartilage, and synovial fluid. An excess production of collagenases can destroy extracellular matrix and lead to pathological conditions (Osathanunkul et al., 2013; Kim et al., 2016). Moreover, several studies have shown that collagenases are biomarkers of various diseases, particularly tumor invasion and metastasis (Seltzer et al., 1990; Efsen et al., 2011). Thus, developing new methods to rapid, sensitive and selective detect type IV collagenase is of great significance in the diagnosis of type IV collagenase-relevant diseases.

The traditional methods that have been used for assessing type IV collagenase activities mainly involve colorimetric method (Osathanunkul et al., 2013), gelatin, enzyme linked immunosorbent assay (ELISA) (Patel et al., 2005; Yoshioka et al., 1987), enzyme-specific substrates (Bickett et al., 1993) and zymography (Snoek-van et al., 2005; Levenson, 1976). However, these methods involve a multistep detection process including incubation, washing and the application of images for the type IV collagenase in signal cell, which make the whole assay to be laborious and time-consuming. Sano et al. have reported methods using capillary gel electrophoresis with laser-induced fluorescence (CGE-LIF) for collagenase activity assays with high sensitivity. However, higher sensitivity needs to detect the reaction products of collagenase (Sano et al., 2004). Each of these reports individually described a great advance in type IV collagenase detection, either in terms of limit of detection (LOD) or type IV collagenase specificity. Rather than focusing on increasing type IV collagenase detection sensitivity, our work here focuses on type IV collagenase discrimination and much more simple detection system using synthetic peptide.

The synthetic peptides are mostly unstructured, and typically consist of 10–50 residues with varied quantity charged/uncharged residues distributed throughout (Fayad et al., 2017). In a previous study, Type IV collagenase has marked substrate specificity for denatured collagen (Patel et al., 2005). Cleavage site specificity of type IV collagenase from collagen, which has been found in human skin, was determined using small synthetic collagenous peptides with varied sequences around

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glycine. These synthetic peptides act as substrates for type IV collagenase as effectively as denatured collagen. More recently, Liu et al. showed that the synthetic peptides, immobilized on quantum dots (QDs), can be used to fabricate a peptide/QDs-based fluorescence resonance energy transfer (FRET) biosensor for the detection of cancer marker type IV collagenase (Liu et al., 2011). However, the prepared processes of CdTe QDs may be complex, and it has certain toxicity when used as bioassay. To resolve this problem, peptides-based quartz crystal microbalance (QCM) may be selected as a useful technique to investigate the activity of type IV collagenase.

Alternatively, QCM is a convenient and useful analytical tool for analysis of biological molecules (Sauerbrey, 1959). Peptide-based QCM biosensors have been constructed for the detection of bacteria in our research group (Dong and Zhao, 2015). The method has some major advantages over other methods: it is relatively simple; as physical/chemical manipulations are minimized; the analytical processes are real-time and label-free; and the exposure to radiation can be avoided relative to photoanalytical chemistry. However, one drawback of this approach is that sensitivity is still dissatisfactory especially for low-mass biological molecules. Signal amplification would be one of the major approaches for developing highly sensitive QCM biosensors. Au nanoparticles (Au NPs) are frequently used for signal amplification in biological molecules analysis because metal nanoparticles have higher-mass and also are easy to functionalize (Chen et al., 2010).

In this work, we developed a highly sensitive QCM biosensor for type IV collagenase detection using Au NPs to enhance frequency signals. The designed peptide (noted as P1) can be bound on the QCM electrode by a thiol of terminal cysteine. While the studied collagenase is presented, P1 will be hydrolyzed into small pieces, and leave from the QCM electrode surface. The specific hydrolyzed process can cause the frequency change of QCM to give the signals of hydrolysis rate related to the concentrations of type IV collagenase. Moreover, the designed peptide contains positive charges coming from arginine, which allow the peptide to bind with the negative functionalized gold nanoparticles. When P1 was tethered to gold nanoparticles (Au NPs/P1), the signal can be amplified to further enhance the sensitivity of the designed biosensor. Under the optimal conditions, the QCM-based biosensor shows excellent sensitivity with a LOD of 0.96 ng mL^{-1}.

2. Experimental

2.1. Materials

Type IV collagenase was obtained from Biodee Biotechnology corporation Ltd (Beijing, China). The designed cysteine-tagged peptide (noted as P1), H-Cys-Ala-Pro-Gly-Gly-Ala-Arg-Arg-Arg-Arg-Arg-NH_2, was purchased from APeptide Co., Ltd (Shanghai, China). The peptide was purified to a signal peak on HPLC and the structure confirmed by mass spectroscopy. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was used as a reducing agent to reduce the disulfide bonds formed in the synthesized peptide. The stock solution of P1 was prepared by the reconstitution of the lyophilized powders in phosphate buffered saline (PBS) (Sigma-Aldrich) consisting of 137 mM NaCl, 2.7 mM KCl, 4.4 mM Na_2HPO_4, 1.4 mM KH_2PO_4 (pH 7.4) and 1.0 mM TCEP (Kulagina et al., 2007). Human serum albumin (HSA) was purchased from Sigma (St. Louis, MO, USA). $HAuCl_4$ $4H_2O$, mercaptoacetic acid (MCA), and 6-mercapto-1-hexanol (MCH) were obtained from Shanghai Chemical Reagents (Shanghai, China). 1,10-phenanthroline was purchased from De En Chemical Reagent (China). All other chemicals were of analytical grade and used without further purification.

2.2. Apparatus

QCM measurements were performed on a quartz crystal microbalance (CHI 440 A, Shanghai Chenhua Ltd. China). A 7.995 MHz AT-cut quartz crystal (13.7 mm diameter) covered with gold electrodes (5 mm diameter) was mounted levelly in a homemade Teflon holder. This Teflon holder was placed in a temperature controlled box, and linked with a six-channel peristaltic pump (BT100K, http://www.crpump.com). Electrochemical impedance spectroscopy (EIS) was carried out with an electrochemical workstation (CHI660C, Shanghai Chenhua Ltd., China). EIS measurements were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution which were purged with highly purified nitrogen for at least 10 min. The experiment temperature was controlled at 37 °C for the type IV collagenase detection.

2.3. Synthesis and functionalization of gold nanoparticles

According to a previously reported method with a minor modification (Zhang et al., 2010), Au NPs were prepared. Briefly, 5 mL of trisodium citrate (1.0%) was added to 100 mL of boiling $HAuCl_4$ solution (0.25 mM) under stirring for 10 min, and the resulting solution was cooled to room temperature. The size of Au NPs obtained (an average size of 13 nm) was evaluated by using a JEM-100CX transmission electron microscope (Tokyo, Japan) (Li et al., 2010). The as-prepared Au NPs were further fabricated with the MCA and therefore it carried negative charge. In brief, Au NPs (5 mL, 20 nM) were incubated with the MCA solution (50 mL, 10 mM) for 1 h under stirring condition. The final mixture was slowly brought up to a final concentration of 10 mM TCEP in PBS (pH 7.4) and allowed to age for 10 h. Centrifugation was performed for 20 min in order to remove excess MCA, subsequently. The precipitate was washed with PBS (pH 7.4) solution, recentrifuged, and finally dispersed in PBS (pH 7.4) containing 0.01 M NaCl for the further use.

2.4. Preparation of the amplified QCM biosensor

Prior to the immobilization procedure, the QCM gold electrode was cleaned firstly with piranha solution (H_2O_2/H_2SO_4 1:3 (v/v)) for 30 s, rinsed with ethanol, and then rinsed thoroughly with water. The cleaned QCM electrode was subsequently installed in a homemade Teflon holder. A simple technique for the immobilization of P1 to the QCM electrode surface is through the reaction between the thiol groups present in cysteine residues and Au. The cysteine residues can be synthetically introduced at a specific terminal site of the P1 to form a properly oriented peptides layer. For the immobilization of P1, a solution containing 200 μg mL^{-1} of P1 in PBS (adjusting pH 10.76 with 0.1 M NaOH) was injected into the QCM Teflon chamber and incubated 6 h under dark and static condition. The isoelectric point (PI 10.76) of P1 in PBS was detected by electrophoresis method. The P1 molecules would be dispersed uniformly in PBS under the isoelectric point condition. The frequency signal response of QCM was used to evaluate the density of P1 on the surface of QCM gold electrode. Then, an aqueous solution of 0.5 mM 6-mercapto-1-hexanol (MCH) was injected into the QCM sensing chamber for 1 h to passivate the sensor surface. After this step, the PBS was adjusted pH to 7.4 in the QCM chamber. At this point, the P1 was positively charged, which is beneficial to bind with the negative functionalized gold nanoparticles. The electrostatic interaction of arginine with positive charge in P1 resulting in the covalent immobilization of P1 on sensor surface via C-terminal cysteine at an angle to the surface (Strauss et al., 2010). A 10 nM MCA functionalized Au NPs solution was injected to the QCM sensing chamber until a stable signal (ΔF) was observed. After each step, the QCM sensing chamber was rinsed by injecting PBS solution (pH 7.4) at a flow rate of 0.2 mL min^{-1} for 5 min to remove the unbound compounds. An amplified QCM chip (Au NPs/P1-QCM electrode) for collagenase detection was obtained. More of the same amplified QCM chips were prepared under the same conditions. In order to estimate electrode-to-electrode repeatability, the measurements of frequency signals of QCM were performed at every step. As a control, P1-QCM chip was also prepared under the same conditions without the modified with Au NPs. These mentioned modified QCM electrodes were stored at −4 °C for the

further use.

2.5. Collagenase activity assay

The sensing chamber with Au NPs/P1-QCM electrode was placed in an incubator to control the examining temperature. The PBS (pH 7.4) was first injected in the Au NPs/P1-QCM biosensor with a flow rate of 0.1 mL min^{-1} and the crystal frequency was then measured as a background signal, simultaneously. After the signal change (ΔF) was less than 0.5 Hz of drift in 1 min, the subsequent experiment would be continued. Under the static condition, the frequency change of the QCM biosensor, in response to the addition of collagenase solutions of varying concentrations, was then recorded over time. For signal amplification comparison, the frequencies of the control QCM biosensors (P1-QCM electrodes) were determined following the same steps. The homologous enzymes such as lysozyme thrombin and trypsin were further studied under the same conditions for investigating the specificity and selectivity of the designed system to collagenase.

3. Results and discussion

3.1. Construction of sensor system

The peptide (P1) for this work was designed and synthesized with Pro-Gly sequence, which can be specific hydrolyzed by type IV collagenase as outlined in Scheme 1. First, P1 was immobilized on a QCM gold electrode to form a peptide monolayer. After the injection of type IV collagenase solution, the frequency signal increases (curve A in inset of Scheme 1) following the cleavage of P1 (Scheme 1A). Even if the viscoelastic properties of QCM have to be taken into account if the absorbed mass is not rigid as in the case of peptide layer, Sauerbrey demonstrated that the resonance frequency relative increase with decrease of mass on the sensor surface, which was described by the Sauerbrey equation [Sauerbrey, 1959; Nirschl et al., 2011; Ozalp, 2011]. After the binding of Au NPs with the P1 (Scheme 1B), the rate of frequency change was significantly amplified under the same conditions due to the cleavage and leaving of larger mass of Au NPs/peptide residues (curve B in inset of Scheme 1). Obviously, Au NPs/P1 can be used as a signal enhancer for highly sensitive detection of type IV collagenase.

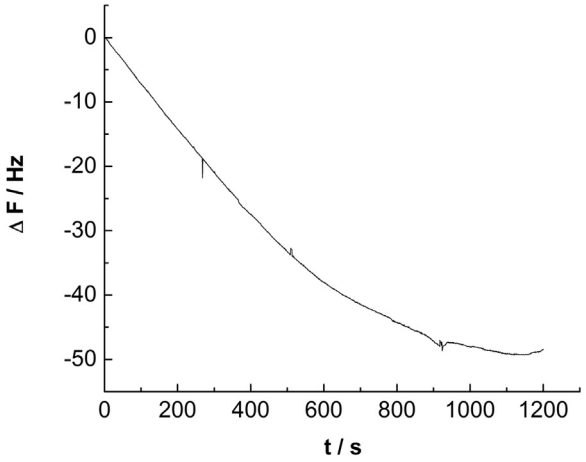
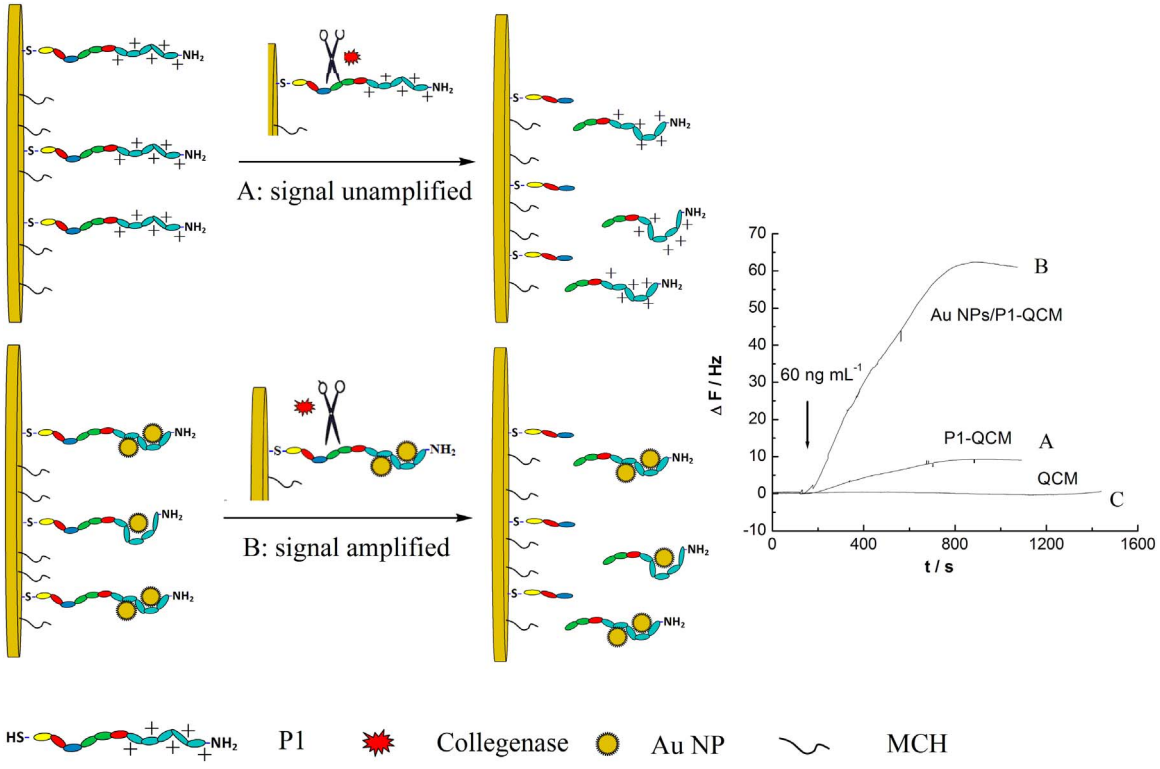


Fig. 1. Characterization of the sensing interfaces by EIS. (a) Bare electrode, (b) after immobilization of P1, (c) incubated with 10 nM Au NPs solution.

3.2. Characterization of sensor system

The coverage of immobilized P1s is crucial to the sensitivity of the Au NPs/P1-QCM biosensor. A cysteine residue was attached at the C-terminal of the P1 to immobilize the peptide on QCM electrode. As shown in Fig. 1, the immobilization was observed by a frequency drop (−48.5 Hz) of QCM when the P1 solution arrived at the chamber. Here, the molecular weight of P1 is 1254.5 g mol^{-1}. According to the Sauerbrey equation, $\Delta f = -C_f \times \Delta m$, where $C_f = 0.142$ Hz (ng $cm^2)^{-1}$ for a 7.995 MHz AT-cut crystal at 20 °C, therefore, the mass loading of immobilized P1 per area is about 4084 ng cm^{-2}, which give a peptide



Scheme 1. Schematic diagram illustrating the comparison between signals unamplified and amplified detection of collagenase based on P1-QCM (A) and Au NP/P1-QCM (B) biosensors.

surface coverage of 8.2×10^{14} molecules cm^{-2}. Wu et al. reported a peptide surface density of 7.4×10^{13} molecules cm^{-2} with the same method as ours (Wu et al., 2010). The larger surface coverage of peptide obtained in our study, which is comparable with literature values, may be due to the smaller size of the peptide we used.

EIS measurements were also employed to investigate the characteristics of the P1-immobilized gold surface. A $Fe(CN)_6^{4-/3-}$ redox couple was used as the probe. In the Nyquist plots of EIS, an increase of the diameter of the semicircle reflects an increase of the interfacial electron-transfer resistance (R_{et}) (Deng et al., 2009). First, The prepared bare Au electrode was rinsed and transferred to a $Fe(CN)_6^{4-/3-}$ solution (5 mM) for EIS measurements. Fig. S1 showed the Nyquist plots of the EIS of bare Au, P1-Au, and Au NPs/P1-Au electrodes in $Fe(CN)_6^{4-/3-}$ solutions. The smallest Ret (~ 0.06 KΩ) was observed for the bare Au electrode (curve a). After the immobilization by P1s, a dramatic increase of Ret (~ 59.5 KΩ) can be found (curve b). These results imply the formation of a dense biomolecular monolayer of immobilized P1s on Au electrode, which impedes the electron-transfer of electrode surface. Further linking with Au NPs resulted in an impedance decrease (~ 43 KΩ , curve c) compared to the unlinked surface. This may be explained that the linking by electrostatic attraction between positively charged P1s and negative Au NPs forms an electroneutral interface, which facilitate an enhanced electron-transfer process of $Fe(CN)_6^{4-/3-}$. These experimental results demonstrate that the sensing interface has been successfully fabricated.

To investigate the fabrication of Au NPs/P1-QCM biosensor and the process of type IV collagenase detection, SEM images were also taken for observing the surface change of bare QCM, P1-QCM, Au NPs/P1-QCM and Au NPs/P1-QCM. Compared with bare QCM (Fig. S2E, inset of Fig. S2A), a flocculated molecular layer was observed (Fig. S2A), which revealed the successful immobilization of P1 into QCM electrode. The coupling of Au NPs with P1 is an important procedure, which plays an important role on the signal amplification for type IV collagenase detection. As can be seen from Fig. S2B, individual Au NPs (white dots) were observed after the binding of Au NPs with P1 on the QCM electrode. Moreover, the performances of Au NPs/P1-QCM for type IV collagenase detection were also investigated using SEM. Two different concentration solution of type IV collagenase (25 ng mL^{-1} and 60 ng mL^{-1}) were tested and the corresponding morphologies were represented in Fig. S2C and D, respectively. The injection of type IV collagenase solution with higher concentration resulted in additional decrease of Au NPs from sensor surface, suggesting correlation between the cleavage rates of P1 and the concentrations of type IV collagenase.

3.3. Sensitivity of amplified Au NPs/P1-QCM biosensor

Using synthetic peptide of the structure P1, we confirmed the earlier report (Weingarten and Feder, 1986) that type IV collagenase cleaves the peptide bond between proline and glycine. In enzyme-catalyzed reaction, the rate of the enzyme catalyzed reactions can be increased by increasing concentration of the enzyme (Ajmala et al., 2016). Because of the P1s were immobilized on QCM electrode, relative rate of cleavage of P1s by type IV collagenase results in the variation of frequency change rate, which related to the concentration of type IV collagenase in matrix (Seltzer et al., 1990). Therefore, the rates of frequency change (ΔF) calculated on the basis of type IV collagenase activity as an internal standard.

3.3.1. Amplification effect of sensing system

Amplification effect of Au NPs was studied subsequently by using a P1-modified QCM electrode. First, the direct detection of type IV collagenase was studied through monitoring the QCM responses induced from P1 cleaved reaction in the presence of type IV collagenase. As illustrated in Fig. 2 (The red curve was a fitted linear curve of signal response of biosensor, which slope indicated a rate of frequency change within 10 min), with the injection of type IV collagenase solution

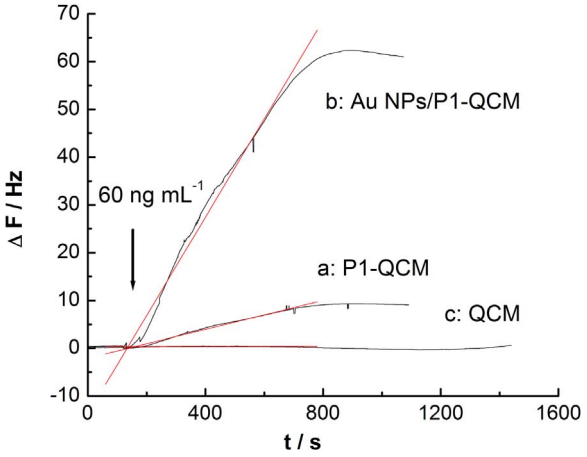


Fig. 2. Signal responses of the P1-QCM (a), Au NPs/P1-QCM (b) and bare QCM (c) biosensors in 60 ng mL^{-1} type IV collagenase solution at 37 °C. The red curve was a fitted linear curve of signal response of biosensor, whose slope indicated a rate of frequency change within 10 min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

(60 ng mL^{-1} final concentration), the frequency shift from 0.11 to 7.6 Hz was observed. The rate of frequency change (ΔF) of QCM was 0.0149 Hz s^{-1} within 10 min. When type IV collagenase concentration was lower than 40 ng mL^{-1}, it was difficult to discriminate the frequency response from the baseline noise (data not shown). When P1-modified QCM chip was replaced by Au NPs/P1-modified QCM chip in the experimental process, the rate of frequency shift obviously increased with the same concentration of type IV collagenase (frequency shifts from 0.06 to 60.1 Hz were observed), as shown in Fig. 2b. The rate of ΔF of Au NPs/P1-QCM biosensor was 0.137 Hz s^{-1} within 10 min, which is 9.19 times compared with the P1-QCM biosensor (Fig. 2a) at same conditions. This could be explained by the fact that Au NPs were introduced as a signal amplified reagent to improve the sensitivity of the biosensor. Simultaneously, a control experiment (monitor QCM signal over time without immobilized of P1 or Au NPs/P1) was shown in Fig. 2c, which revealed that the rate of frequency change of biosensor over time was minimal and did not impact on the measurements. Moreover, the performances of Au NPs/P1-QCM biosensor under the different surface coverage of P1were investigated. The results showed that the optimized saturated surface coverage of P1 for the type IV collagenase detection was 8.2×10^{14} molecules cm^{-2}.

3.3.2. Au NPs/P1-QCM amplified biosensor for type IV collagenase

The Au NPs/P1-QCM biosensor was fabricated to determine the activity of type IV collagenase with different concentrations of type IV collagenase (0–180 ng mL^{-1}). As shown in Fig. 3, increasing the concentration of type IV collagenase from 10 to 60 ng mL^{-1} resulted in a significant rate increase of the frequency change from 0.00851 to 0.137 Hz s^{-1} (define as the slope of red curve, which is the fitted linear curve of signal response of biosensor within 10 min after sample injection). However, further increasing the concentration of type IV collagenase (from 70 to 180 ng mL^{-1}) can only cause a moderate rate increase of ΔF (from 0.209 Hz s^{-1} to 0.832 Hz s^{-1}), indicating that the immobilized P1s were almost all cleaved away from the Au NPs/P1-QCM biosensor surface within 400 s. QCM assays are very convenient and sensitivity for type IV collagenase detections. Nevertheless, it has been noticed that the rates of QCM frequency change are not linear with type IV collagenase concentrations. Fig. 4 shows the logarithm of rate of ΔF (log[rate(ΔF/s)]) as a function of the concentration of type IV collagenase (range from 10 to 180 ng mL^{-1}). A linear relationship between the log[rate(ΔF/s)] and the concentration of type IV collagenase was obtained in a range from 10 to 60 ng mL^{-1}. The linear relationship can be described as $y = -2.28 + 0.024x$ with a correlation coefficient of

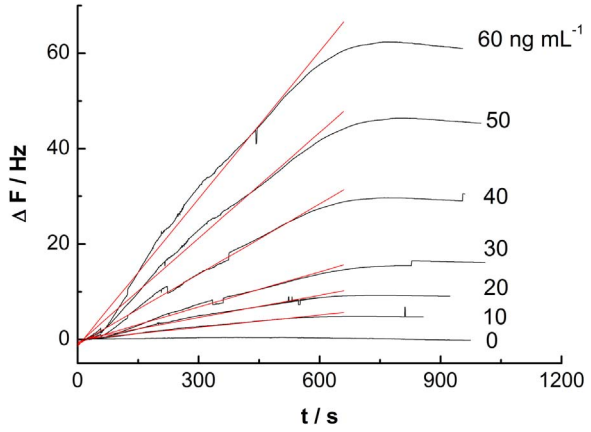


Fig. 3. Real-time signal responses of the Au NPs/P1-QCM biosensor in 0–60 ng mL^{-1} type IV collagenase solution at 37 °C. The red curve was a fitted linear curve of signal response of biosensor, whose slope indicated a rate of frequency change within 10 min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

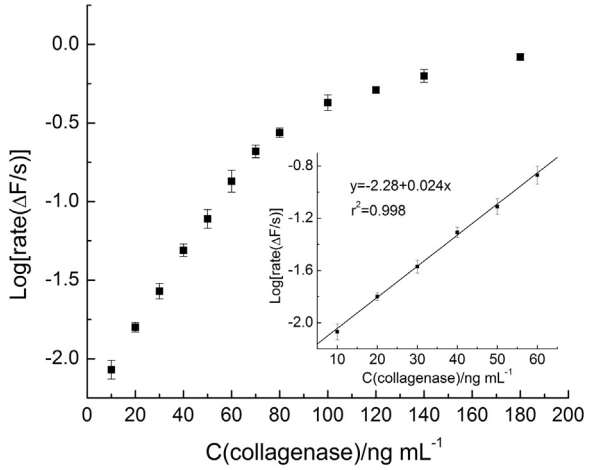


Fig. 4. The values of log[rate(ΔF/s)] of the Au NPs/P1-QCM biosensor to different concentrations of type IV collagenase from 10 ng mL^{-1} to 180 ng mL^{-1}. Inset: the linear relationship between the logarithm of rate of ΔF (log[rate(ΔF/s)]) and the concentration of type IV collagenase in the range of 10–60 ng mL^{-1}. The error bars represent the standard deviation (S.D.) of three measurements.

0.998, where y is the logarithm of rate of ΔF (log[rate(ΔF/s)]) and x is the concentration of type IV collagenase. The limit of detection (LOD, defined as the average blank value plus 3 times the value of standard deviation of the blank value, converted to type IV collagenase concentration (Wu et al., 2010)) of the Au NPs/P1-QCM biosensor for type IV collagenase was 0.96 ng mL^{-1}. The LOD is much lower than that achieved with the quantum dots-based FRET biosensor (limit of detection, 18 ng mL^{-1}) (Liu et al., 2011), the quantum dot-based proteolytic sensor (limit of detection, 82.8 ng mL^{-1}) (Díaz et al., 2015), as well as this obtained with a ninhydrin-based spectrophotometry (limit of quantification, 10 ng mL^{-1}) (Zhang et al., 2013). As shown in Fig. S3, if the direct detection of type IV collagenase was performed by the P1-QCM, a linear range of 40–120 ng mL^{-1}, and LOD of 21 ng mL^{-1} were obtained (Fig. S4). An enhanced sensitivity of 6.5 times was found by comparing the Au NPs/P1-QCM and P1-QCM biosensor. This demonstrates that the proposed Au NPs/P1-QCM biosensor can be employed for type IV collagenase activity highly sensitive detection. Furthermore, the response time of biosensor is a significant parameter (Peng et al., 2017). With regard to Au NPs/P1-QCM biosensor, the reaction equilibrium time can be decreased (less than 800 s) by increasing concentration of type IV collagenase and the rate of the enzyme

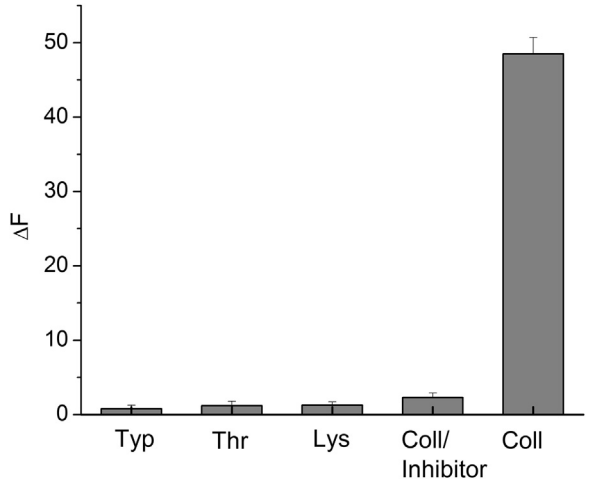


Fig. 5. Specificity of the Au NPs/P1-QCM amplified biosensor, gray columns represent the relative frequency responses within 10 min. In blank, PBS buffer (pH 7.4) was injected instead of collagenase; the concentrations of trypsin; thrombin and lysozyme were all 5 μg mL^{-1}; the collagenase solution (60 ng mL^{-1}) containing 1,10-phenanthroline (0.1 μg mL^{-1}, a metalloprotease inhibitor); the concentration of collagenase was 60 ng mL^{-1}, respectively.

catalyzed reactions is linear change within 600 s.

3.4. Specificity of the Au NPs/P1-QCM amplified biosensor

In order to explore the specificity of the Au NPs/P1-QCM biosensor, three kinds of homologous enzymes such as lysozyme, thrombin and trypsin were further studied under the same conditions. The frequency change of Au NPs/P1-QCM was recorded when the biosensor immersed in trypsin (5 μg mL^{-1}), thrombin (5 μg mL^{-1}) lysozyme (5 μg mL^{-1}) or collagenase (60 ng mL^{-1}) solutions, respectively. As shown in Fig. 5, less than 2 Hz frequency change can be observed implying non-cleaving activity of the Au NPs/P1 amplified QCM biosensor for trypsin, thrombin and lysozyme (compared to a 48.6 Hz frequency change when the solution containing 60 ng mL^{-1} of type IV collagenase was injected to the same system). Furthermore, in order to verify the cleave reaction between P1 and type IV collagenase, a metal chelator 1,10-phenanthroline, which is often used as a metalloprotease inhibitor, was chosen. To determine its inhibition of type IV collagenase, a solution of 1,10-phenanthroline was made by dissolving in ethanol, followed by dilution to 0.1 μg mL^{-1} with PBS solution. When a type IV collagenase solution (60 ng mL^{-1}) containing 1,10-phenanthroline (0.1 μg mL^{-1}) was injected in Au NPs/P1 amplified QCM biosensor, just a 2 ± 0.5 Hz frequency change was observed, demonstrating that the activity of type IV collagenase was inhibited in presence of 1,10-phenanthroline.

3.5. Application of the method to spiked samples

Since the detection of type IV collagenase in serum is clinically relevant, the ability of the Au NPs/P1 amplified QCM biosensor to detect the analyte in complex matrixes was tested. For dilution fold of 5, 10, 20 and 30, the obtained recoveries for type IV collagenase (25 ng mL^{-1}) in spiked serum were 113%, 107%, 98% and 103% with corresponding relative standard deviation (R.S.D., n = 3) of 11%, 9%, 10% and 5%, respectively. These results suggest that the Au NPs/P1 amplified QCM biosensor exhibits satisfactory analytical ability towards type IV collagenase in complex matrixes.

4. Conclusions

A signal amplified type IV collagenase QCM biosensor was developed utilizing the small synthetic peptide and Au NPs. The amplified

signal response and specificity of the Au NPs/P1 QCM biosensor originates from the immobilization of high mass Au NPs to synthetic peptide and the specific hydrolyzed cleavage of the peptide bond between proline and glycine by type IV collagenase. The QCM biosensor, Au NPs/P1-QCM, exhibited high selectivity, sensitivity, fast response, and feasibility in complex matrixes. We anticipated that the methodology would be extended for some fields such as clinical diagnostic, environmental, and food analysis. Furthermore, this study displays a novel idea in virtual screening type IV collagenase inhibitors, and may be potentially suitable for designing or screening inhibitors of other targets.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2018.01.069.

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