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Electrochemical impedance spectroscopy for quantization of matrix Metalloproteinase-14 based on peptides inhibiting its homodimerization and heterodimerization

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ABSTRACT

We reported here two novel electrochemical impedance spectroscopy biosensors were developed for the first time for highly sensitive quantification of matrix metalloproteinase-14 (MMP-14) based on binding interaction between hemopexin-like domain (PEX) of MMP-14 (PEX-14) and its inhibitory peptides. Specific inhibitory peptides (IVSC or ISC) inhibiting homodimerization or heterodimerization of MMP-14 was first self assembled on the surface of gold electrode and blocked with 6-mercapto-1-hexanol on a gold electrode surface used as IVSC or ISC modified biosensor, respectively. IVSC modified biosensor can be used for detection of MMP-14 by using the direct IVSC-MMP-14 interaction inhibiting MMP-14 homodimerization as well as ISC modified biosensor for indirect detection of MMP-14 via PEX-14 mediated peptide-MMP-14 binding. The electron transfer resistance (R_{et}) of biosensor was monitored to measure MMP-14 using $Fe(CN)_6^{3-/4-}$ as probe. The increase of the R_{et} of the biosensors are linear with the concentration of MMP-14 in the range from 1 μg L^{-1} to 10 μg L^{-1} with detection limit of 0.19 μg L^{-1} for IVSC modified biosensor and 0.1 ng L^{-1} to 50 ng L^{-1} with detection limit of 7 ng L^{-1} for ISC modified biosensor. This work demonstrates that probing the interaction between peptide inhibitor and PEX of MMPs represents a novel approach to assess MMPs-mediated cancer dissemination.

1. Introduction

Matrix metalloproteinases (MMPs) are a group of enzymes that are responsible for cell migration, invasion, proliferation, and apoptosis [1]. It has been found that MMPs are mainly overexpressed in pathological conditions including vascular disease, cancer progression, bone disorders, and neurodegenerative diseases [2]. MMP-14, a membrane type-1 MMP (MT1-MMP) is a key protein in cancer invasion and metastasis [3]. The previous reports demonstrated that only MMP-14 can promote cellular invasiveness into collagen I matrix in epithelial cells and fibroblast [4]. Additionally, MMP-14 can activate pro-MMP-2 and pro-MMP-13, which further enhances the proteolytic repertoire characteristic of invasive cancer cells [4]. It has been proposed as important biomarker of infection and inflammation [5]. Hence, the development of sensitive and precise MMP-14 sensing technologies is highly relevant for cancer-related research, including cancer theragnosis and drug screening.

Assays for the detection of MMPs for both clinical and research purposes are summarized as following. Enzymatic, immunochemical and fluorimetric methods are commonly used techniques in clinical research [2,6]. In vivo imaging methods are of particular interest in cancer research and diagnostics [7]. Immunochemical methods precede enzymatic methods, but cannot distinguish between active MMP and inactive MMP in zymogene form [6a]. There are a number of other methods that are subjects of interest such as western-blotting [8], enzyme-linked immunosorbent assays (ELISA) [9], mass spectroscopy [10], liquid chromatography [11], zymography [12] and surface plasmon resonance [13] assays. Many novel biosensors or biosensing methods based on hydrolytic cleavage of MMP-specific peptides were used to detect MMPs, such as MMP-9 [14], MMP-3 [15], MMP-7 [16] and MMP-2 [17]. These peptides were selected by the catalytic domains of MMPs which share high amino acid similarity. As a consequence, distinguishing between MMPs is extraordinarily difficult [18]. Therefore, analysis of unique features of individual MMPs is necessary need for the clinical diagnosis.

The peptide inhibiting MMP-9 was selected using hemopexin-like

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(PEX) domain of MMP-9 as model by Bjorklund's group for the first time. Different from catalytic domains of MMPs, the inhibitory peptide own high specificity [1]. Zucker group observed that synthesized inhibitory peptides interfere with MMP-14 homodimer and hereodimer with CD44 based on specific interaction between inhibitory peptides and PEX domain of MMP-14 (PEX-14), respectively [3]. To our best knowledge, inhibitory peptide specifically binding PEX domain of MMP, which is crucial for many specific MMP functions, has not been employed as the recognition elements for detection of MMPs. It follows that synthesized peptides selected by PEX domain can be expected to be further promoted and applied.

Electrochemical Impedance spectroscopy (EIS) becomes increasingly popular for electrochemical measurements of molecular interactions and is widely used in a variety of biosensing applications [19]. EIS biosensors, which are based on the change of electron transfer resistance using a redox probe couple such as $[Fe(CN)_6]^{3-/4-}$, have received much attention due to high sensitivity [19]. There are a growing number of publications on the use of EIS for antibody/antigen [20], DNA or aptamer [21], cell or bacteria [22].

Herein, taking advantage of the EIS biosensor and specific binding between inhibitory peptide and PEX domain of MMP, we reported a simple label-free electrochemical biosensor for highly sensitive and selective detection of MMP for the first time. In this work, MMP-14 was chosen as a model protein. PEX-14 is necessary for MMP-14 homodimerization and heterodimerization with CD44 [3,23]. The thiolated (by incorporating with a cysteine) PEX-14 binding peptides (named ISC and IVSC), which inhibit severally MMP-14 heterodimerization with CD44 and homodimerization, are designed as recognition elements according to Zucker group [3]. The label-free electrochemical biosensors of MMP-14 are depicted in Scheme 1. Scheme 1 (A) displays a direct MMP-14 detection by IVSC-MMP-14 binding event. Scheme 1 (B) shows an indirect MMP-14 detection based on PEX-14-mediated ISC-PEX-14-MMP-14 binding. The direct or indirect MMP-14 detection biosensor was fabricated by self-assembling a thiolated ISC or IVSC on the surface of a gold electrode and blocking with 6-mercapto-1-hexanol (MCH). For the Scheme 1 (A), different concentrations of MMP-14 directly bind to IVSC on gold based on interaction between IVSC and PEX-14 of MMP-14 inhibiting MMP-14 homodimerization [3]. For the Scheme 1 (B), fixed concentration of PEX-14 saturate ISC on the gold surface inhibiting MMP-14 heterodimerization with CD44 and then bind to different concentrations of MMP-14 based on MMP-14 homodimerization dependent on blades IV of the PEX domain [3]. The changed signals of the two biosensors are gathered from EIS. A systematic study of this new biointerface using these two different transduction mechanisms provided complementary information, that can be used for cross-validation of the data in addition to have enhanced sensitivity, selectivity and linear range.

2. Materials and methods

2.1. Chemicals and materials

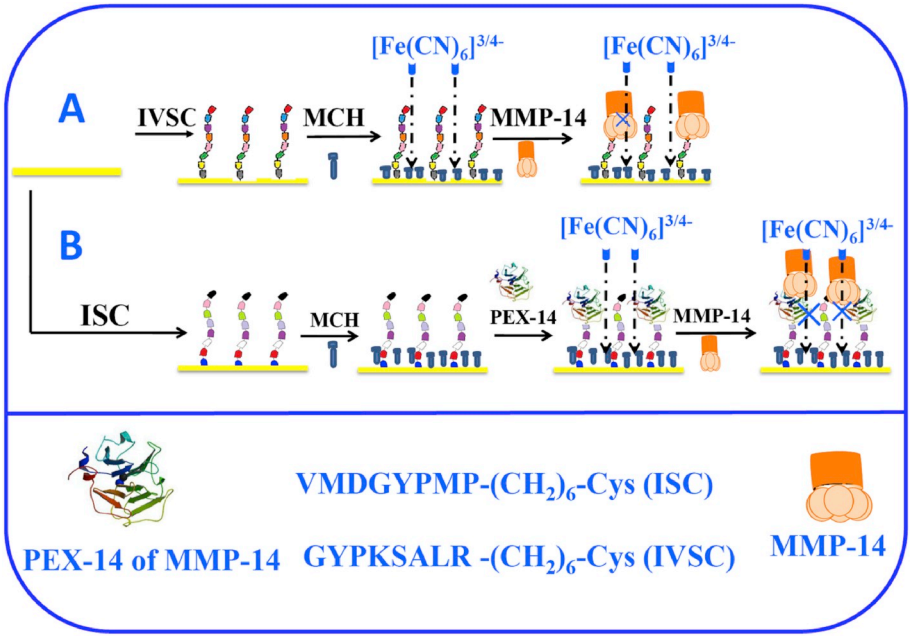
Tris (hydroxymethyl) aminomethane (Tris-HCl) was purchased from Shanghai Macklin Biochemical Co., Ltd; Potassium hexacyanoferrate (III) was from Sinopharm Chemical Reagent Co., Ltd; Potassium chloride was purchased from Guangdong Guanghua Sci-Tech; Sodium nitrate was purchased from Guangdong Guanghua Sci-Tech Co., Ltd; Acetonitrile was purchased from Sigma-Aldrich Co., Ltd; Recombinant human PEX-14 protein (PEX-14) and MMP-14 were purchased from Abcam Co., Ltd; Thrombin and Trypsin was purchased from Sigma-Aldrich Co., Ltd; MMP-2 and MMP-7 was purchased from Protech Co., Ltd.; Deionized water was used throughout the experiments.

The eight-amino peptides, VMDGYPMP (IS4) and GYPKSALR (IVS4), were reported in Ref. [3]. The designed peptide was used in this work. The peptides would be labeled "$(CH_2)_6$-Cys-OH" so that the peptides can be immobilized on the gold electrode. Peptides sequences, VMDGYPMP (IS4)–$(CH_2)_6$–Cys-OH (abbreviated ISC) and GYPKSALR (IVS4)–$(CH_2)_6$–Cys-OH (abbreviated IVSC), were synthesized by Shanghai Apeptide Co., Ltd.

The frabrication of biosensors and electrochemical measurements are presented in the Supporting Information.

3. Results and discussion

PEX domains of all MMPs have pseudo-four-fold symmetry [3]. The fourth outer strand (S4) of PEX domains MMP selectively interact with other specific protein components [24]. The structure of isolated MMP-14 PEX domain protein (abbreviated PEX-14) was characterized by x-



Scheme 1. Schematic Representation of Different Modes of MMP-14 Detection Used in This Work: (A) a Direct MMP-14 Detection Using IVSC-MMP-14 Binding and (B) a PEX-14-Mediated MMP-14 Detection Using ISC-PEX-14-MMP-14 Binding.

ray crystallography and observed that bulges in the outermost strands of blades I and IV in PEX domain, which are unique to MMP-14 [23]. Zucker group found that MMP-14 homodimerizes through blade IV and interacts with CD44 through blade I of the PEX domain and further observed that synthesized inhibitory peptides IVS4 and IS4 interfere with MMP-14 homodimer and hereodimer with CD44, respectively [3]. That means inhibitory peptides IVS4 and IS4 dimerized with blades IV and I of the PEX-14 domain, respectively. Our latest work designed non-label and labeled biosensor for PEX-14 detection using inhibitory peptide ISC used as recognition element [25]. Our study results indicated that the inhibitory peptide for PEX domain can be used recognition elements for MMPs determination. In this work, two novel biosensors for detection of MMP-14 were fabricated using inhibitory peptides selected by PEX domain as recognition which are different from previous works using substrate peptides selected by catalytic domain (CAT) of MMPs.

3.1. EIS biosensor for direct detection of MMP-14 via IVS4-MMP-14 binding

3.1.1. Performance of EIS biosensor for direct detection of MMP-14

The electrodes in each fabrication step were characterized by EIS and cyclic voltammetry (CV) as shown in Fig. S1 (see the Supporting Information). All observations of EIS and CV suggest that biosensor is successfully fabricated and can be used for the following quantitative performance. For direct detection of MMP-14, this direct binding of MMP-14 to IVS4 modified gold electrode leads to a change at the electrode interface as discussed in above. Fig. 1 shows the R_{et} changes when MMP-14 at different concentrations was consecutively added to the cell. After the experimental impedance data were fitted using the above equivalent circuit model [26], we can be observed from the fitting of measured spectra (line) that R_{et} increased with increasing concentration of MMP-14 introduced into the detections chamber. With increasing concentrations of MMP-14, the R_{et} goes increasing as well which indicates a signal ON approach. By using this approach, we were able to detect MMP-14 in a different concentration range, i.e., 1 $\mu g\,L^{-1}$ to 10 $\mu g\,L^{-1}$. The linear regression equation was $R_{et} = 0.3591\,C + 15.76$ (unit of C is $\mu g\,L^{-1}$, $R = 0.99$) with a detection limit calculated according to S/N = 3 as 0.19 $\mu g\,L^{-1}$. Theoretically speaking, the analysis should be a much broader range than what was shown here, as EIS is a signal ON sensor which provides more space for signal growth with increasing concentrations. The possible reason for the smaller concentration range is that the short sequence of IVS4 modified on the gold surface is relatively weak and flexible which provides high stereospecific blockade for the binding MMP-14. Therefore, we should seek the second type of binding event by tight and rigid binding via PEX-14 mediated sandwich assay.

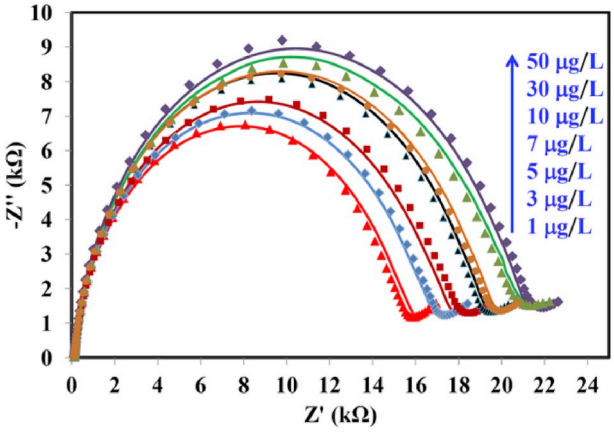


Fig. 1. Nyquist plots of impedance spectra of direct detection MMP-14 EIS biosensors fabricated after interaction with different concentrations of MMP-14 for 40 min.

3.2. EIS biosensor indirect detection of MMP-14 via ISC-PEX-14-MMP-14 binding

As discussed above, a binding on the biosensor surface which is not rigid cannot provide true quantitative information for MMP-14 detection. Thus the key principle in this measurement is to ensure adequate MMP-14 binding by using recognition molecules with high affinity and multiple binding sites. MMP-14 mainly consist of four distinct domains, which are N-terminal pro-domain, catalytic domain (CAT), hinge region, and C-terminal hemopexin-like domain (PEX) [1,27]. PEX domain of MMP-14 (PEX-14) is necessary for MMP-14 homodimerization and heterodimerization with CD44. In detail, blade IV of PEX-14 is necessary for MMP-14 homodimerization, while blade I is required for heterodimerization with CD44 [3,23]. Corresponding synthetic peptide inhibitors, IVSC and ISC, interfere with MMP-14 homodimer and hereodimer with CD44 [3]. Therefore, the binding event, ISC-PEX-14-MMP-14, can be deduced and served as recognition system for detection of MMP-14. Take note of ISC in place of IVSC in the following study. If IVSC was used to bind PEX-14, the PEX-14-MMP-14 interaction would be interfered. For this new interface, these ISC-PEX-14-MMP-14 interactions are proved even more beneficial, widening both the limits of detection as well as the range of detection. For this purpose, we used PEX-14 as an MMP-14 adhesion promoter to strongly attach MMP-14 to the ISC modified electrode so a rigid binding layer would be formed on the electrode surface depicted in Scheme 1 (B).

3.2.1. Characterization of PEX-14 binding with ISC modified gold electrode

The electrodes in each fabrication step were characterized by EIS and CV as shown in Fig. S2 (see the Supporting Information). The EIS and CV confirmed that the biosensor was successfully fabricated. And then, with addition of PEX-14 and MMP-14 to the biosensor surface in turn, the R_{et} increased, which indicated that the fabricated EIS biosensor can be used to detect MMP-14 via PEX-14 mediated. To examine the ISC modified gold electrode specificity, PEX domain of MMP-2 (PEX-2), PEX domain of MMP-7 (PEX-7), thrombin and BSA, were selected as negative controls. As shown in the Fig. S3 (A), there were negligible R_{et} changes for the addition of PEX-2, PEX-7, thrombin and BSA. This result shows that the ISC modified sensor is antigen specific. Fig. S3 (B) shows the R_{et} changes when PEX-14 at different concentrations was consecutively added to the cell. Initially this R_{et} increased corresponding to more concentration of PEX-14 bound to the ISC modified gold electrode, and then reached a plateau at 10 $ng\,mL^{-1}$. Clearly, at the relatively high concentration, the binding of PEX-14 to the surface bound ISC approaches saturation. This study demonstrates that the ISC modified sensor has high sensitivity and specificity for binding to the PEX-14, which are corresponding to our latest results [25]. 10 $ng\,mL^{-1}$ of PEX-14 was used for the following study.

3.2.2. Performance of EIS biosensor for indirect detection of MMP-14

The experimental conditions were optimized as shown in Fig. S4. And then, the determination of MMP-14 by the biosensor was performed. Before the measurements of MMP-14, the ISC modified gold surface was first immersed into PEX-14 solution with concentration of 10 $ng\,mL^{-1}$ for 30 min and used as PEX-14/ISC modified sensor. And then, MMP-14 samples with concentrations from 0.1 $ng\,L^{-1}$ to 50 $ng\,L^{-1}$ were poured into the PEX-14/ISC modified sensor chambers and measured by EIS. Fig. 2 showed the obtained results. The linear regression equation was $R_{et} = 0.8151\,C + 24.08$ (unit of C is ng/L, $R = 0.9938$) with a detection limit calculated according to S/N = 3 as 7 $ng\,L^{-1}$.

In order to compare the performance of two EIS biosensors, calibration curves between signals and concentrations of MMP-14 were drawn as shown in Fig. 3. Fig. 3 shows the well fitted linear curves both from IVSC (a) and PEX-14/ISC (b) interface respectively. From the Fig. 3, the following key conclusions can be summarized: (1) From the

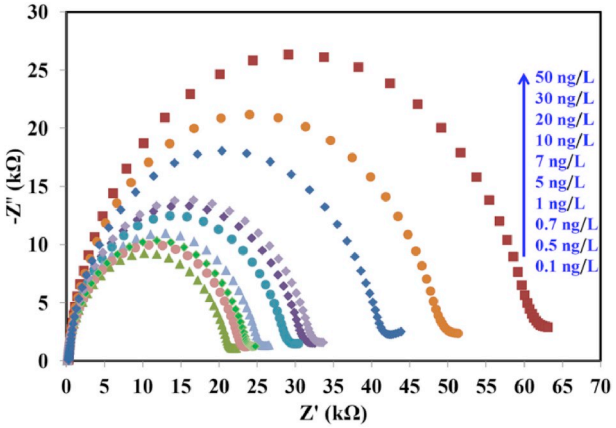


Fig. 2. Nyquist plots of impedance spectra of indirect detection MMP-14 EIS biosensors fabricated after interaction with different concentrations of MMP-14 for 40 min.

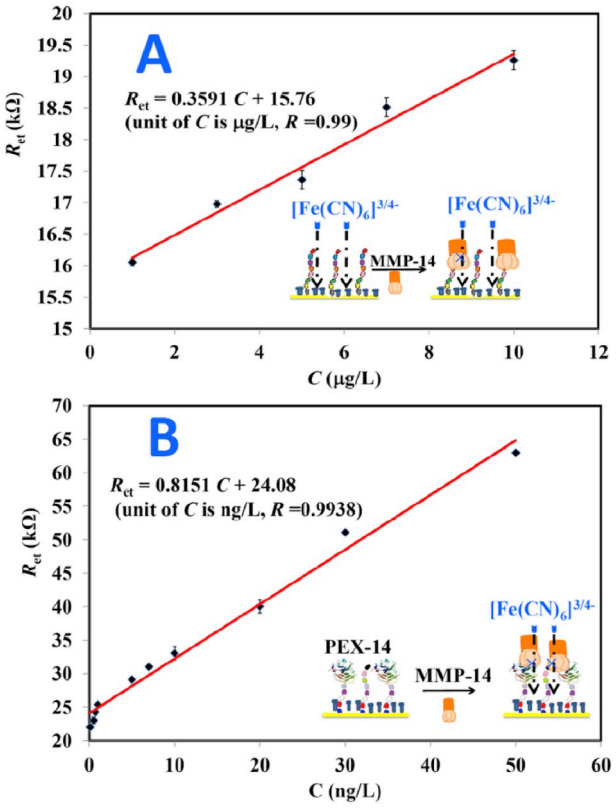


Fig. 3. Calibration curves of sensor signals drawn against concentrations of MMP-14, where (A) direct detection of MMP-14 through IVSC-MMP-14 binding (B) indirect detection of MMP-14 via ISC-PEX-14-MMP-14 binding while.

comparison of (a) and (b) curves, it can be observed that the signals changed are much higher for the PEX-14/ISC interface and the detection limit for this interface is 27 fold higher than the IVSC modified surface, which resulted from the rigid binding events of PEX-14 mediation as discussed above. (2) Additionally, it can be found that the linear ranges of the two biosensors are quite different. The linear range from 0.1 ng L^{-1} to 50 ng L^{-1} for the PEX-14/ISC interface is broader than the range from 1.0 μg L^{-1} to 10 μg L^{-1} for the IVSC interface. From this comparison, we can conclude that the PEX-14/ISC interface enhanced the assay range. (3) Finally, in comparison to PEX-14/ISC sensor, the IVSC modified sensor exhibited worse sensing performances, but both the two biosensors are much better than previously reported biosensors or biosensing methods used for detection of MMPs. Table 1 lists the detection results obtained from the reported biosensors or biosensing methods based on antibody or cleaved peptides. From the Table 1, the detection limits of the two EIS biosensors are lower than that obtained from the reported sensing methods, such as 20 μg L^{-1} MMP-7 from mass spectrometry [10], 0.92 μg L^{-1} MMP-9 obtained from microfluidic device of fluorescence detection [14b], and 0.68 μg L^{-1} MMP-14 obtained from fluorescence assay [15]. And the detect limits obtained from our work are even lower than 0.6 μg L^{-1} of MMP-3 obtained from a antibody-based SPR biosensor [28]. The lower detection limits and broader assay range gained from the two interfaces designed in our work may be owed to the following reasons. (1) compared to the traditional ELISA methods based on antibody as molecular recognition element [29] and novel sensing methods on basis of hydrolytic cleavage of MMP-specific peptides [14b], the simple biosensor in our work used the inhibitory peptides as biorecognition elements can overcome the disadvantages of antibody and hydrolytic cleavage of MMP-specific peptides, such as, large size, limited target analytes, limited shelf lives and temperature-sensitive to undergo denaturation (for antibody) and non-specifically absorption and result in false positive signal (for hydrolytic cleavage of MMP-specific peptides). (2) The rigid binding of ISC-PEX-14-MMP-14 is used to enhance the sensitivity of the biosensor.

3.2.3. Selectivity of EIS biosensor

Selectivity is a necessary parameter for evaluation of the biosensor. In order to check the biosensor only response to MMP-14, MMP-7, MMP-2, thrombin and trypsin were used (as shown in theFig. 4). Very small changed signals were obtained for the tested MMP-7 and MMP-2. Negligible signal changes were detected from the thrombin and trypsin. Good selectivity of this biosensor was evident.

3.2.4. Real sample analysis of the proposed biosensor

In order to assess the practical applicability of proposed biosensor, a recovery experiment was carried out by adding four various concentrations of MMP-14 into 30-fold-diluted healthy human serum. As shown in Table S1, the acceptable recoveries (ranged from 116% to 101%) suggested the promising potential application in clinical detection.

4. Conclusion

In this work, we showed an integrated approach to address the concerns of inhibitory peptides selected by PEX domain of MMP based biosensors for MMP-14 detection with the following new strategies: (1) using inhibitory peptides modified biointerface fabrication for direct MMP-14 detection via IVSC-PEX-14; (2) enhancing MMP-14 recognition by combining it with an additional binding event, i.e. ISC-PEX-14-MMP-14 binding, thereby enhancing the sensitivity and limit of detection; The sensor fabricated in this way showed the lower detection limits of 0.19 μg L^{-1} (for direct detection IVSC-PEX-14 binding) and 7 ng L^{-1} (for indirect detection ISC-PEX-14-MMP-14 binding), as well as better selectivity and stability as compared to the presently available technologies. The EIS biosensors could be applied as an ideal sensing platform for the detection of other different types of cancer biomarkers by changing the corresponding molecular recognition substance, laying the foundation for cancer diagnosis and prognostic evaluation.

Notes

The authors declare no competing financial interest.

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Table 1
Comparison of techniques for the detection of MMPs.

Refs	MMP	Assay principle	DL	Linear ranges
10	MMP-7	Time-of-flight Secondary Ion Mass Spectrometry	20 μg L^{-1}	2–2 × 10^4 μg L^{-1}
17b	MMP-2	Electrochemiluminescence Biosensor	33 fg L^{-1}	1 × 10^{-4} − 1 × 10^2 μg L^{-1}
28	MMP-3	SPR Biosensor	0.6 μg L^{-1}	21.5–2.15 × 10^3 μg L^{-1}
30	MMP-2	Fluorescence Resonance Energy Transfer Based Biosensor	0.01 μg L^{-1}	0.01–0.5 μg L^{-1}
14b	MMP-9	Microfluidic Device for Fluorescence Detection	0.92 μg L^{-1}	0–9.2 μg L^{-1}
17a	MMP-2	Electrochemical Detection using PtNPs as Scaffolds	0.03 ng L^{-1}	0.1–20 μg L^{-1}
15	MMP-2	Metal Enhanced Fluorescence Assay	12.2 pg L^{-1}	1 × 10^{-2} − 100 μg L^{-1}
	MMP-3		60 ng L^{-1}	0.1–500 μg L^{-1}
	MMP-7		0.22 pg L^{-1}	1 × 10^{-2} − 100 μg L^{-1}
	MMP-9		102 pg L^{-1}	1 pg/mL − 100 μg L^{-1}
	MMP-14		0.68 μg L^{-1}	1 μg L^{-1} − 100 μg L^{-1}
This work	MMP-14	Direct EIS biosensor	0.19 μg L^{-1}	1 − 10 μg L^{-1}
	MMP-14	Indirect EIS biosensor	7 ng L^{-1}	0.1–50 ng L^{-1}

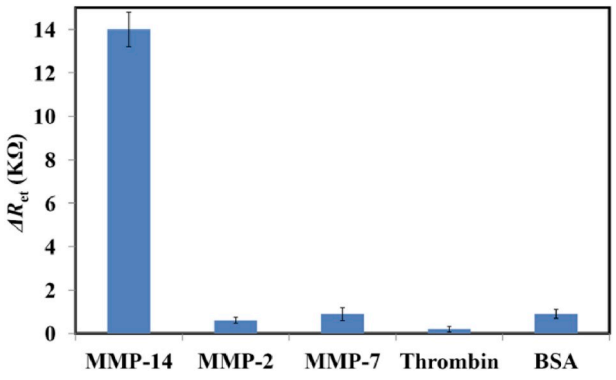

Fig. 4. EIS impedance changed signals of the direct detection MMP-14 EIS biosensors after individual interaction with 7 μg L^{-1} MMP-14 or 60 μg L^{-1} representative interfering proteins including MMP -7, MMP-2, thrombin and trypsin.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.120142.

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