Journal of
Materials Chemistry B

Accepted Manuscript

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View Article Online
DOI: 10.1039/C8TB02894J

Journal Name

ARTICLE

A novel ratiometric SERS biosensor with one Raman probe for ultrasensitive microRNA detection based on DNA hydrogel amplification

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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In this work, a novel ratiometric SERS biosensor with only one Raman probe combining with DNA hydrogel-captured glucose oxidase (GOx) amplification method were fabricated to realize accurate and sensitive assay for microRNA (miRNA) 122, which could overcome complex operation process and chemical waste of traditional ratiometric approach with two different signal probes. Here, 3-mercaptophenylboronic acids (3-MPBA) as a standard reference with unique Raman peak at 996 cm^{-1}, was firstly connected on the silica@Au nanoflowers (Si@AuNFs) SERS substrates. When the DNA hydrogel containing GOx was opened by released DNA (R) from the target miRNA induced cycle amplification process, the GOx would be released from DNA hydrogel. Therefore, the GOx can catalyze glucose to produce H_2O_2 on the Si@AuNFs. Soon afterwards, the acquired H_2O_2 can further react with 3-MPBA to produce 3-hydroxythiophenol (3-HTP) with a new Raman peak at 883 cm^{-1}. During this time, the peak intensity of 996 cm^{-1} almost remain the same which can act as reference standard. And the intensity ratio of 883 cm^{-1} to 996 cm^{-1} is increased with the increasing concentration of target miRNA 122, thus achieving quantitative detection of miRNA 122. As a consequence, our SERS biosensor can sensitively detect miRNA 122 from 10 aM to 100 pM and the detection limit was 7.75 aM. Our strategy adopts a novel ratiometric method with one Raman probe to detect miRNA, opening a new avenue for trace amount of biological sample detection with high sensitivity and accuracy.

Introduction

MicroRNAs (miRNAs), as a class of non-coding RNA species, play crucial roles in a variety of biological processes.[1-3] It is critical to rapidly and sensitively detect miRNAs because they were known as various biomarkers of human cancers.[4,5] Surface enhanced Raman spectrum (SERS) possess superiorities of rapid response, nondestructive detection and high sensitivity and so on, which is often used for detection of miRNA. [6-8] Although excellent performance can be obtained with this sensitive technique, some difficulty is still existed to obtain repeatable signal with a same SERS substrate. This phenomenon may cause inaccurate quantification of target analyte.[9,10] Therefore, a reliable ratiometric SERS biosensor is proposed to overcome the above problems.

Traditional ratiometric SERS sensor always utilized two different signal probes to achieve quantitative detection of target, [11-13] which could improve the accuracy and credibility of SERS assay. However, the use of two probes also has some drawbacks such as the complexity of labeling procedure and expense of the chemicals. Herein, only one Raman probe 3-mercaptophenylboronic acid (3-MPBA) is used for construction of new ratiometric method to achieve sensitive detection of target with simple operation and low cost. 3-MPBA is a boronate molecule, which selectively and efficiently responses to H_2O_2, producing its corresponding phenol form.[14] Due to the special catalytic ability of H_2O_2, 3-MPBA can be oxidized to 3-hydroxythiophenol (3-HTP), leading to obvious differences of SERS spectrum. This unique boronate-to-phenol switch can cause a new Raman peak of 883 cm^{-1} which belongs to benzene ring stretching.[15,16] In this time, the peak of 996 cm^{-1} owing to the C-C in-plane bending will not change.[17] Therefore, 996 cm^{-1} can be an internal standard, which assist the peak of 883 cm^{-1} as detection label of analyst.

DNA hydrogel as a regulable and smart DNA structure was applied widely in the field of bioassay for the advantages of great biocompatibility and sensitive controllability.[18-20] Recently, we have designed Raman probe toluidine blue (TB) captured DNA hydrogel as an amplification method to achieve detection of miRNA 155.[21] However, the amount of TB packaged in the DNA hydrogel was limited for the finite capacity of hydrogel, which confined the further improvement of sensitivity for bioassay. In this work, we utilized DNA hydrogel to capture glucose oxidase (GOx). When miRNA 122 existed, the DNA hydrogel structure would be destroyed to release GOx from hydrogel to catalyze glucose for producing H_2O_2, which can further oxidize 3-MPBA to 3-HTP to obtain sensitive Raman

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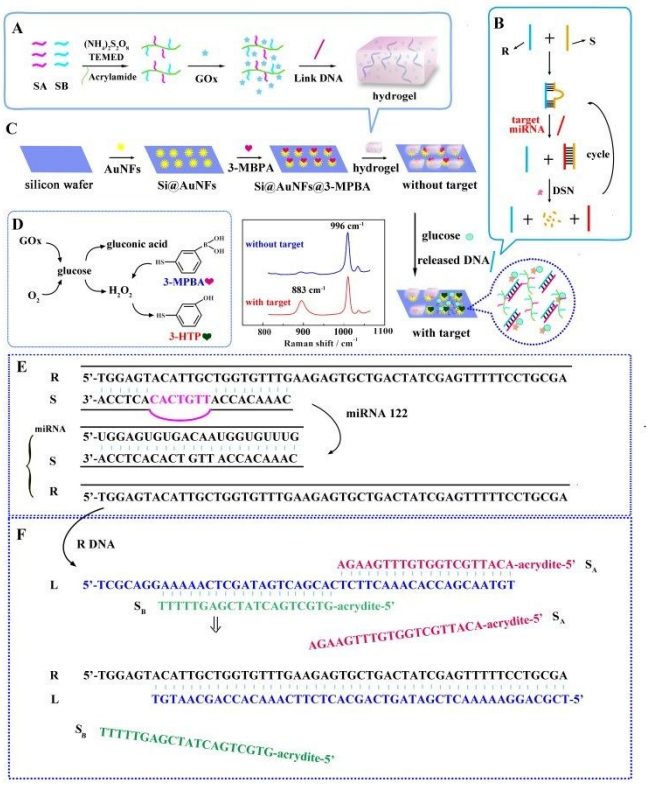
signal. This efficient enzyme catalytic system can obtain lots of 3-HTP to obviously improve the sensitivity of SERS biosensor.

In this work, a cascade catalytic reaction was utilized to detect miRNA 122 with ratiometric method. Firstly, 3-MPBA can connect with Au nanoflowers (AuNFs) to generate a unique peak at 996 cm^{-1}. At the same time, glucose oxidase (GOx) as a high-efficiency H_2O_2-generating catalyst was trapped in the DNA hydrogel and thus the catalytic ability was closed for the shingling of hydrogel. However, the DSN enzyme induced cycle reaction can produce abundant released DNA (R DNA) to open the DNA hydrogel with the presence of miRNA 122. By this way, the GOx would be released on the SERS substrates (Si@AuNFs@3-MPBA) producing H_2O_2 to catalyze 3-MPBA to 3-HTP. During this time, the Raman peak of 883 cm^{-1} belonged to 3-HTP would appear and increase for the adding of H_2O_2. At this time, the peak intensity of 996 cm^{-1} almost remained constant in this conversion process. By this way, the peak intensity ratio at 883 cm^{-1} and 996 cm^{-1} would positively correlate with the logarithm of target miRNA 122 concentration. And the assembly process of the SERS biosensor and principles of catalysis were shown in Scheme 1. Based on this sensitive ratiometric method and significant amplification capability of DNA hydrogel technology, the designed SERS biosensor can sensitively detect miRNA with a good performance which have great potential to be applied in clinical diagnostics.



Scheme 1. (A) The synthesis possess of GOx wrapped DNA hydrogel; (B) MiRNA 122-induced signal amplification process; (C) The construction process of the SERS biosensor; (D) Cascade catalytic reaction process; (E) DNA strand displacement reaction between R, S and target miRNA; (F) DNA strand displacement reaction between R, L, SA and SB DNA.

Results and Discussion

Materials Characterization

The TEM of AuNFs was shown in Figure 1A. From Figure 1A, the AuNFs presented obvious flower structure with size of about 100 nm. The corresponding UV-Vis and DLS data of Au NFs was presented in Figure S1. Figure 1B was the gradual modification process of the SERS biosensor. Firstly, 3-MPBA as an internal standard was connected on the AuNFs through Au-S bond to present a strong peak at 996 cm^{-1} (curve a). At this time, the peak of 883 cm^{-1} was very weak. After glucose was added on the Si@AuNFs@3-MPBA, the peak of 996 cm^{-1} did not change for glucose would not catalyze 3-MPBA as shown in curve b. When GOx wrapped DNA hydrogel was added without the target miRNA, the intensity of Raman peak was weakened which presented as curve c. This may be due to the covering effect of hydrogel. However, when the GOx was released from DNA hydrogel in the presence of miRNA 122, the GOx can catalyze glucose to generate H_2O_2. Then H_2O_2 can further react with 3-MPBA to produce 3-HTP with an obvious Raman peak at 883 cm^{-1} (curve d). The corresponding SEM images of each process were shown in Figure S2. And the corresponding chemical formula was shown in Figure 1C. The DNA hybrid situation of strand displacement reactions used in this work was also shown in scheme 1E and 1F.

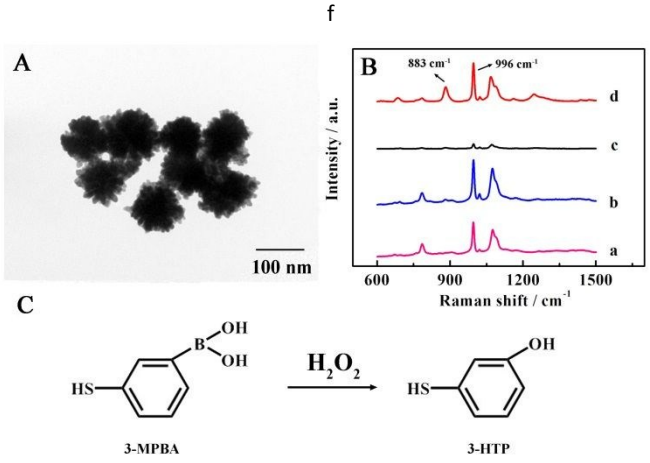


Figure 1. (A) TEM images of Au nanoflower; (B) SERS spectrum of (a) Si@AuNFs@3-MPBA; (b) Si@AuNFs@3-MPBA after glucose was added on the SERS substrate; (c) SERS of Si@AuNFs@3-MPBA@glucose after GOx trapped DNA hydrogel; (d) SERS of Si@AuNFs@3-MBPA@GOx@glucose after the products from miRNA induced DSN cycle was added; (C) The chemical process of 3-MPBA to 3-HTP by the oxidizability of H_2O_2.

DOI: 10.1039/C8TB02894J

Optimization of Experimental Conditions

In this work, the concentration of DSN enzyme, incubation time of target miRNA 122 and the concentration of GOx trapped in the DNA hydrogel play important roles in improving the performance of SERS biosensor. Exploring the optimal experiment condition is necessary and significant. As depicted in Figure 2A, the ratio of Raman intensity (I_{883} / I_{996}) increased accompany with the increasing DSN concentration until achieving a plateau. This phenomenon indicated that 0.03 U was the best concentration of DSN chosen in this experiment. Similarly, 40 min was chosen to as optimal incubation time in this experiment for the experiment efficiency can achieve maximum value (Figure 2B). And Figure 2C, the optimal concentration of GOx trapped in DNA hydrogel was 2.0 mg mL^{-1} (Figure 2C).

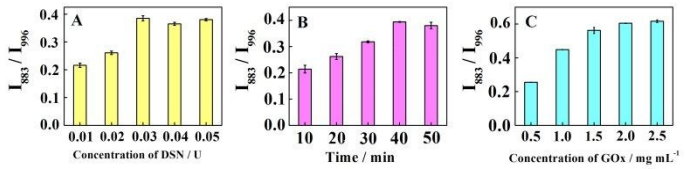


Figure 2. The ratio of Raman intensity (I_{883} / I_{996}) (A) with different concentration of DSN on the SERS biosensor; (B) with different incubation time of miRNA 122; (C) with different concentration of GOx trapped in the DNA hydrogel on the SERS platform.

Performance of the SERS Biosensor

Under the optimized conditions, the relationship between the Raman intensity and concentration of target miRNA 122 was illustrated in Figure 3A. Obviously, accompanied with the increasing concentration of miRNA 122, the intensity of 883 cm^{-1} increased correspondingly. However, the peak of 996 cm^{-1} remained unchanged during this time which cause the ratiometric peak intensity (I_{883} / I_{996}) increased accordingly. As a result, the ratiometric peak intensities of I_{883} / I_{996} is linear related with the logarithm concentration of miRNA 122 between 10 aM to 100 pM. The detection limit is 7.75 aM. The regression equation is y = 0.048lg$c$ + 0.149 (where y is the ratio of the Raman intensity between the peak of 883 cm^{-1} and 996 cm^{-1}, c is miRNA 122 concentration), which was presented in Figure 3B. The corresponding correlation coefficient square is 0.997. The error bar was calculated by testing each sample 3 times. Also, this cascade catalytic ratiometric SERS biosensor was compared with other works to detect miRNA 122 (Table 1). We can see that our biosensor displayed better performance.

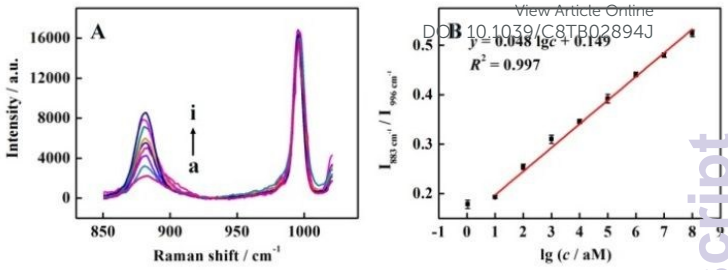


Figure 3. (A) SERS spectra of the ratiometric SERS biosensor with different concentration of miRNA 122 (a - i: 0 - 100 pM). (B) The calibrat of ion plot of I_{883} / I_{996} *vs* lg$c$.

Table 1. Comparison Result the ratiometric SERS biosensor with otherworks to detect miRNA 122

Method	Detection limit	Linear range	Reference
Fluorescence	5 nM	5-1000 nM	22
Electrochemistry	0.1 fM	0.1 fM-500 pM	23
Chemiluminescence	0.82 fM	1 fM-100 pM	24
Chemiluminescence	10 fM	10 fM-1 nM	25
SERS	0.24 pM	1 pM-1 nM	26
SERS	7.75 aM	10 aM-100 pM	This work

Selectivity

Some interfering substances such as miRNA 155, miRNA 141, miRNA 21, one base mismatch DNA, three bases mismatch DNA (100 pM) and their complexes (including 100 pM three DNAs and 100 fM miRNA 122) were separately tested by this GOx-trapped DNA hydrogel based biosensor. As shown in Figure 4, when the interferents were added, insignificant changes could be found compared to that with no target miRNA. When the mixture containing 100 fM target miRNA 122, the ratio was similar with that the value only contained miRNA 122. These results indicated that this GOx captured DNA hydrogel based ratiometric SERS biosensor can detect miRNA 122 with high specificity. The corresponding sequences of DNAs and miRNAs were listed in Table S2.

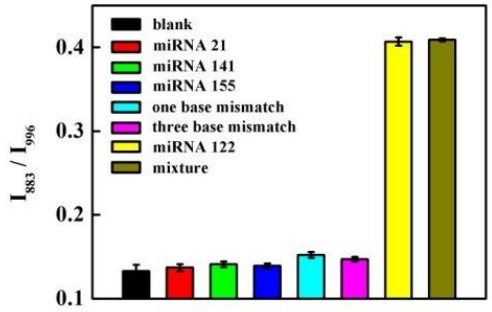


Figure 4. The ratio of Raman intensity (I_{883} / I_{996}) of the proposed SERS biosensor with blank, interferents and mixture solution. The concentration of miRNA 141, miRNA 21, miRNA 155, one base mismatch and three base mismatch DNA were 100 pM and the concentration of miRNA 122 was 100 fM. The mixture contains miRNA 21, miRNA 141, miRNA 155 (the three DNAs are 100 pM each) and miRNA 122 (100 fM).

Reproducibility

20 different points of SERS spectra on a same substrate were collected as shown in Figure 5A. From Figure 5A, we can easily get the RSD of the ratio of Raman peak at 883 and 996 cm^{-1} belong to 20 different points was 0.53%, exhibited a good uniformity of SERS substrate. Meanwhile, 6 different SERS substrates were tested with same conditions to investigate reproducibility of the proposed SERS sensor. From Figure 5B, we can get the RSD was 0.59%, which showed a good reproducibility.

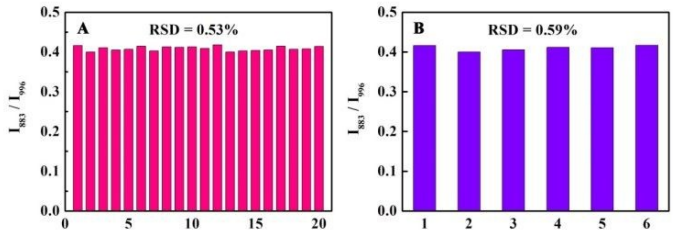


Figure 5. (A) Corresponding histogram for the ratio of I_{883} / I_{996} from 20 different spots on; (B) Histogram for the ratio of I_{883} / I_{996} from 6 different SERS substrates ($c_{miRNA\ 122}$ = 100 fM).

Application.

The reliability of this ratiometric SERS strategy was also explored by adding different concentrations of miRNA 122 into 100-fold diluted healthy human real serum sample. From Figure 6, good recoveries were obtained from 96.13% to 103.4%, which indicated this sensitive SERS biosensor can potentially applied in clinic for detection of miRNA 122.

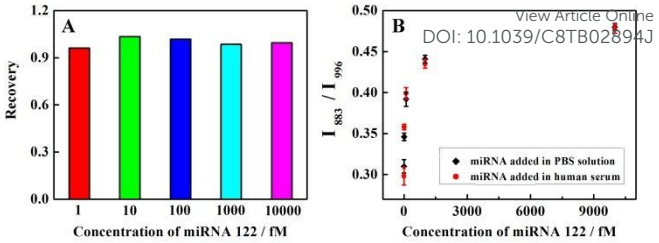


Figure 6. Corresponding (A) histogram recovery results of the proposed method in human serum and (B) plot of I_{883} / I_{996} vs *c*.

Conclusion

In conclusion, a ratiometric SERS biosensor based on GOx captured DNA hydrogel was successfully proposed for miRNA 122 detection. For the high-efficiency cascade catalytic reaction, the proposed strategy owns advantages of high sensitivity, selectivity and accuracy to detect miRNA 122. The fantastic performance was resulted from the following reasons. Firstly, this ratiometric method with one Raman probe can effectively improve the accuracy of the SERS sensor. Secondly, GOx as a high-speed H_2O_2-generating catalyst can obviously improve the reaction efficiency of the biosensor. Thirdly, DNA hydrogel as a switchable structure can flexibly control the trapping and releasing of GOx, thus increasing the controllability and sensitivity of the SERS biosensor. Besides, miRNA 122 induced DSN cycle can effectively amplify detection signal to improve the sensitivity of the biosensor. In a word, this strategy provided a new ratiometric method for sensitive detection of miRNA, holding great promise for reliable detection by SERS method in clinical application.

Acknowledgements

This project has been financially supported by the NNSF of China (21775124， 21675129， 21575116), the Fundamental Research Funds for the Central Universities, China (XDJK2018AA003), China Postdoctoral Science Foundation (2015M572427) and Chongqing Postdoctoral Research Project (xm2015019).

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A ratiometric SERS biosensor with DNA hydrogel-captured glucose oxidase amplification method were fabricated to detect microRNA 122.