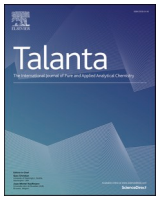
Talanta 245 (2022) 123444

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta



Rolling circle amplification assisted dual signal amplification colorimetric biosensor for ultrasensitive detection of leukemia-derived exosomes

Chao Li [a], Mengyang Zhou [a], Haoyu Wang [a], Jie Wang [b], Lin Huang [a,*]

[a] School of Life Sciences, Anhui Medical University, Hefei, Anhui, 230032, China
[b] Clinical Laboratory Anhui NO.2 Provincial People's Hospital, Hefei, Anhui, 230000, China

ARTICLE INFO

Keywords:
Rolling circle amplification
Colorimetric biosensor
Dual signal amplification
Exosmes
Leukemia

ABSTRACT

Tumor-derived exosomes as a liquid biopsy marker hold great promising for the accurate tumor diagnosis. However, the visual exosomes detection method with high sensitivity and convenience is still a challenge and highly valuable in the clinical application. Herein, we fabricated a colorimetric biosensor that could visually detect leukemia-derived exosomes, and dual amplified the signal based on the rolling circle amplification (RCA) assisted platform. To avoid the interference of components in serum, trigger-aptamer complex was firstly modified on the magnetic beads (MBs) to recognize exosomes, and then release trigger probe to initiate RCA reaction. Therefore, the trigger probe could produce long repeated sequences and hybridized with numerous signal probe. After that, GNPs-HRP (HRP modified gold nanoparticles) bound with signal probe and induced dramatic color change of TMB to achieve the sensing of exosomes. Benefiting from the RCA assisted dual signal amplification, the presented method showed a limit of detection as low as 100 particles/μL. Moreover, this method exhibited good specificity to distinguish healthy and leukemia patients, suggesting its great potential for clinical diagnosis.

1. Introduction

Leukemia is a kind of heterogeneous hematologic malignancy with a high incidence rate and mortality rate. Although the early diagnosis and cancer surveillance is beneficial to improve the five-year survival or overall survival rate, the current diagnosis method mainly relies on bone marrow aspiration which is insensitive and invasive [1]. In contrast, liquid biopsy demonstrated great potential for leukemia diagnosis with noninvasiveness and high sensitivity features through analyzing biomarkers in body fluid, such as circulating tumor DNA (ctDNA) [2], CTC (circulating tumor cell) [3], cell-free nucleic acids (cfNAs) [4], exosomes [5] and so on [6]. So, it is very important to establish a diagnostic method based on liquid biopsy biomarkers for leukemia diagnosis.

Exosomes, as nanoscale extracellular vesicles with the diameter of 30–150 nm, are secreted by most all cells [7]. Based on the mechanism of exosomes formation, biological molecules of parental cells (proteins, RNA, DNA, amino acids, and metabolites) could specifically be packaged into exosomes and play an important role by transferring them into recipient cells [8]. In addition, those components could also predict the state of parental cells [9,10]. So, exosomes have been considered as an important biomarker for liquid biopsy of tumor. Compared with other biomarkers, exosomes also demonstrated many advantages, such as high concentration, good specificity and stability in the liquid body [11]. However, the concentration of exosomes in the early stage of disease is very low. Thus, the sensitivity of method for exosomes detection is very important for early diagnosis of tumor.

In the recent years, several components of exosomes have been shown to have potential as diagnostic disease markers, such as protein [12], miRNA [13], DNA [14] and metabolites [15]. Thus, numerous methods have also been developed to detection exosomes by targeting different markers in exosomes [16–18]. Among them, the detection of miRNA, DNA and metabolites usually need to damage exosomes, and thus resulting in the poor accuracy of exosomes detection [19]. In contrast, the detection of exosomal protein could avoid the damage for exosomes due to it is located on the surface of exosomes. Thus, protein demonstrates better feasibility as marker on exosomes. For the quantification of protein on exosomes, the present methods usually utilize Western blot, mass spectrometry, enzyme-linked immunosorbent assay, flow cytometry and immuno-electron microscopic. However, those methods with low sensitivity and specificity are time-consuming and expensive [20]. Recently, although the microfluidic platform [21], surface plasmon resonance [22], surface enhanced Raman scattering

* Corresponding author.
E-mail address: huanglin8904@163.com (L. Huang).

https://doi.org/10.1016/j.talanta.2022.123444
Received 14 February 2022; Received in revised form 27 March 2022; Accepted 2 April 2022
Available online 12 April 2022

[23], electrochemistry [24] and fluorescence [25] have also been used to enhance analytical performance of exosomes, it is still needed to develop a more convenient and sensitive method for the exosomes detection in clinical application.

Herein, we constructed a dual signal amplified colorimetric biosensor for exosomes detection through integrated RCA and gold nanoparticles. In this work, nucleolin as a biomarker found on the leukemia cells derived exosomes in our previous work was used as target to achieve the sensing of leukemia-derived exosomes [25]. Firstly, the magnetic beads (MBs) were utilized to immobilize the complex of trigger probe and aptamer probe. Thus, the magnetic separation could efficiently avoid the interference of other components in the following steps. In the presence of exosomes, trigger probe could be released through the strong affinity of aptamer with nucleolin, and triggered RCA reaction on the magnetic beads, and then produced numerous repeated sequences to hybridize with signal probe. Lastly, GNPs-HRP bound to the signal probe induced the color change of TMB. Therefore, highly sensitive visual exosomes detection could be achieved through utilizing RCA and GNPs-HRP to induce dramatic color change of TMB. Moreover, the biosensor also displayed a good antijamming capability and performance for the analysis of clinical samples, and showed great potential for the liquid biopsies of leukemia.

2. Experimental section

2.1. Materials and reagents

Human leukemia cell line HL-60 was purchased from the Cell Bank, Chinese Academy of Sciences (Shanghai, China). Chloroauric acid ($HAuCl_4{\cdot}3H_2O$) and Tris (2-carboxyethy) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (StLouis, MO, USA). Sodium citrate and streptavidin-HRP were obtained from Sangon Biotech (Shanghai, China). The oligonucleotide used in this study were synthesized and purified by Sangon Biotech (Shanghai, China), and the sequences are as follows:

Aptamer probe: 5′-CGATGGGTGGTGGTGGTTGTGGTGGTGGTGGT-3.

Trigger probe: 5′bio-TTTTTACCACAACCACCACCACCCATCG-3.

Padlock 1: 5′phosphate-TGGTGGTGGTATGTCCATCAAACTTCT TCCTTGCTGAGGTGTCCGATGGG-3.

Padlock 2: 5′phosphate-GGTGGTGGTATGTCCATCAAACTTCTT CCTTGCTGAGGTGTCCGATG-3.

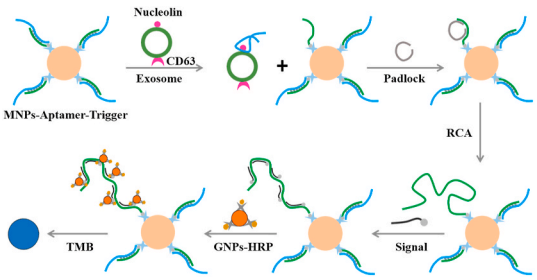
Signal probe: 5′bio-TTTTTTTTTTTCCATCAAACTTCTTCCTTGC.

2.2. Cell culture and exosomes isolation

HL-60 cells were cultured in RPMI with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin at 37 °C under a 5% CO_2 atmosphere. HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with high-glucose, and other conditions are same with HL-60 cells. For the collection of exosomes, the culture medium of cells was replaced with fresh RPMI or DMEM containing exosomes-free FBS after cells grew up to 70% confluency. For another 48 h, the supernatant of cells medium was collected and processed as following step. Firstly, the culture supernatant was centrifuged at 1000 g for 5 min to remove the intact cells. Then, the cell debris and large vesicles were removed by 2000 g for 10 min and 10 000 g for 30 min, respectively. The exosomes were obtained by centrifugation at 100 000 g for 120 min. Finally, exosomes were obtained by ultra-centrifuged at 120 000 g at 4 °C for 120 min.

2.3. The characterization of exosomes

After isolation, the size distribution and concentration of exosomes were firstly detected by nanoparticle tracking analysis (NTA, NanoSight NS300). Then, the markers (CD63 and TSG101) and shape of exosomes were detected by WB and Transmission electron microscopy (TEM), respectively.



Scheme 1. Schematic illustration of RCA induced dual signal amplification platform for exosomes detection.

2.4. The preparation and characterization of streptavidin-HRP modified gold nanoparticles (GNPs-HRP)

To achieve streptavidin-HRP modified gold nanoparticles, the GNPs were firstly prepared by a previous method, and the detailed steps as follows. After heated to boil, 1% sodium citrate (1 mL) was rapidly added into 0.01% $HAuCl_4$ (50 mL) under strong stirring. To ensure the size of GNPs is uniform, the mixture should further be stirred for another 5 min after the solution become into red. Following, streptavidin-HRP was added to 1 mL GNPs solution for 1 h at 37 °C, and continue incubated for another 1h with 10% BSA (100 μl). Lastly, the solution was centrifuged at 10 000 g for 10 min, and then resuspend in 100 μl PBS.

2.5. Exosome detection

For exosmes detection, 10 μl trigger probe (2 μM) was firstly added into 5 μl magnetic beads and incubated in 100 μl PBS buffer for 30 min at room temperature. Then, 10 μl aptamer probe (2 μM) was used to hybridize with trigger probe on magnetic beads after blocking with 100 μL PBS containing 2% BSA and 10 pmoles biotin. To achieve sensing, exosomes with different concentrations were added and reacted for 30 min at room temperature. After incubation, the supernatant was discarded through magnetic separation and washed 3 times with PBS. Next, 100 μl T4 buffer containing padlock probe (10 μl, 2 μM) and T4 DNA ligase (10 U) were added into the above-mentioned magnetic beads for 60 min at room temperature. Following, RCA was performed by adding Phi 29 DNA polymerase (20 U) and 0.5 mM dNTPs in 1 × RCA buffer for 60 min at room temperature. After that, signal probe was added to hybridize with RCA products, and GNPs-HRP was further used to bind with signal probe. In order to produce sensing signal, TMB and H_2O_2 solution was added to achieve color change, and the absorbance value was measured at the wavelength of 650 nm.

2.6. Clinical sample analysis

Human plasma samples from five leukemia patients and five healthy donors were provided by the First Affiliated Hospital of Anhui Medical University (Hefei, China). All experiments were performed in compliance with the relevant laws and institutional guidelines (Biomedical Ethics Committee of Anhui Medical University, China), and informed consent was obtained for any experimentation with human subjects. In order to remove excess large vesicles, the plasma samples were firstly centrifuged at 10 000 g for 10 min. Then, the obtained supernatant (40 μl) was detected according to the above procedure.

3. Results and discussion

3.1. The detection principle of the presented biosensor

The mechanism of the colorimetric biosensor for exosomes detection was illustrated in Scheme 1. In this work, nucleolin protein highly expressed on the HL-60 cells derived exosomes was used as our target.

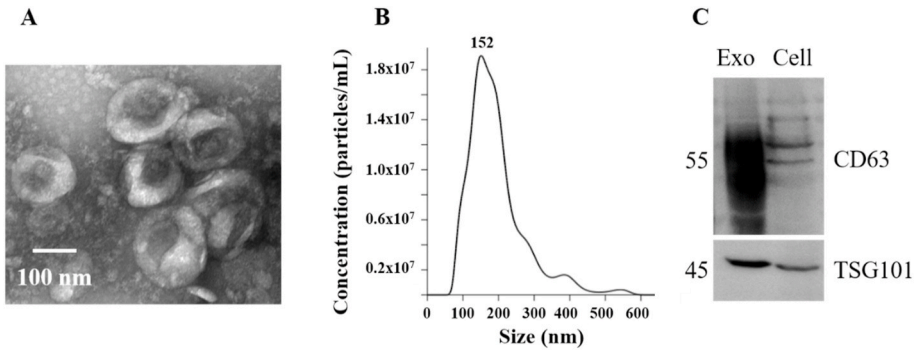


Fig. 1. The characterization of exosomes. (A) TEM image of exosomes derived from HL-60 cells. (B) Nanoparticle tracking analysis (NTA) of purified exosomes. (C) Western blotting image of TSG-101 and CD63 proteins for the HL-60 cells and exosomes lysates.

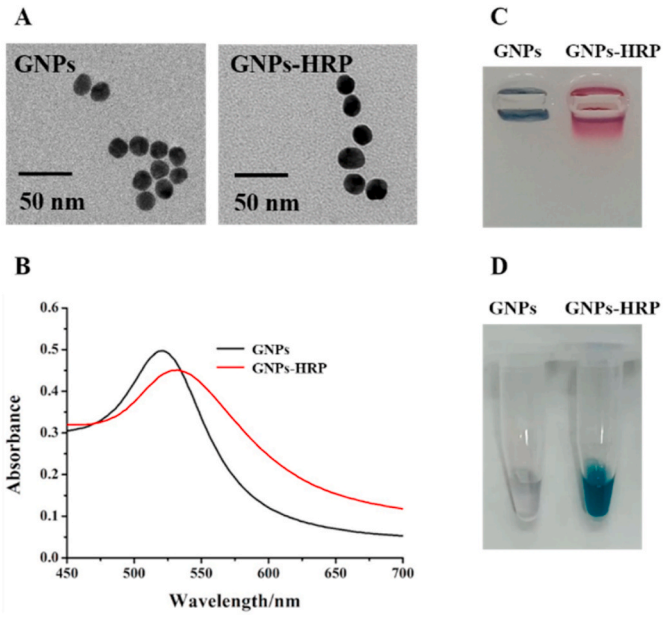


Fig. 2. The characterization of GNPs-HRP. (A) TEM image of GNPs and GNPs-HRP. (B) The UV–vis absorption spectra of GNPs and GNPs-HRP. (C) Agarose gel electrophoresis analysis of GNPs and GNPs-HRP. (D) The color intensity of TMB induced by GNPs and GNPs-HRP in the presence of H_2O_2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

So, the trigger probe was firstly functionalized on the MBs by the interaction of streptavidin and biotin, and then hybridized with aptamer of nucleolin to form complexes. In the presence of exosomes, aptamer bound with exosomes, and then released trigger probe due to the high affinity between aptamers and exosomal nucleolin protein. Therefore, the trigger probe can be further hybridized with padlock probe and extended to form numerous repeated sequences through RCA reaction. After magnetic separation, signal probe modified with biotin at the 5′ end hybridized with RCA products to form double strands, and thus binding with HRP functionalized GNPs to cause a dramatic color change in the presence of TMB and H_2O_2. Finally, UV–vis absorption of above solution was monitored with a UV-spectrophotometer. According to the relationship between absorption value and exosomes concentration, a visual biosensor can be obtained for the convenient and sensitive exosomes detection.

3.2. Characterization of exosomes and HRP functionalized gold nanoparticles (GNPs-HRP)

To obtain the standard samples of leukemia-derived exosomes, multistep differential ultracentrifugation was used to isolation the exosomes derived from the cell culture supernatant of HL-60 cells. Afterwards, the exosomes were analyzed by TEM, WB, and NTA. As shown in Fig. 1A, the shape of exosomes demonstrated typical "cup-shaped" with the size in the range of 100–200 nm. Simultaneously, we also employed NTA to analyze the size distribution and concentrations of exosomes. The result demonstrated the average size was about 152 nm, and the concentration was about 2×10^9 particles/mL (Fig. 1B). To further prove the successful isolation of exosomes, the exosome markers, such as CD63 and TSG-101 protein, have also been detected by WB (Fig. 1C). Therefore, these results suggested that we have successfully obtained exosomes.

To enhance the detection signal of exosomes, we used HRP functionalized GNPs through the interactions between GNPs and mercapto

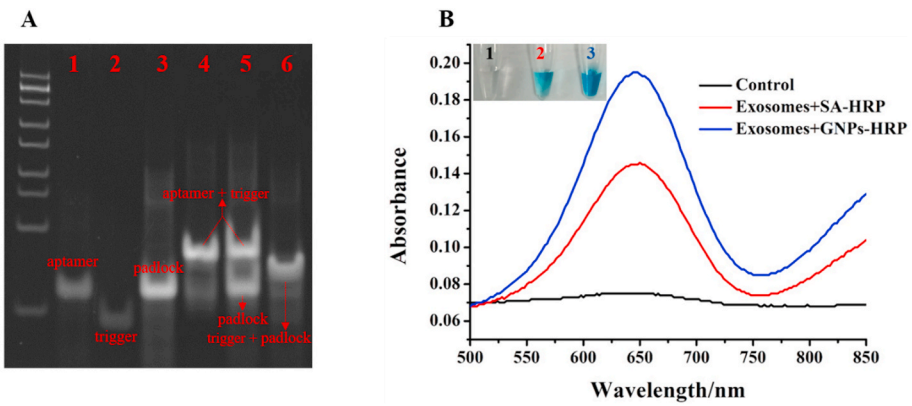


Fig. 3. Feasibility of exosome detection based on the RCA induced dual signal amplification. (A) Native PAGE images of different samples. Lane 1: aptamer, Lane 2: trigger, Lane 3: padlock, Lane 4: aptamer + trigger, Lane 5: aptamer + trigger + padlock, Lane 6: trigger + padlock. (B) The UV–vis absorption spectra of color signal corresponding to the absence (black curve) of exosomes, and the presence of exosomes without (red curve) or with (blue curve) signal amplification (insert is the photo of color intensity). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

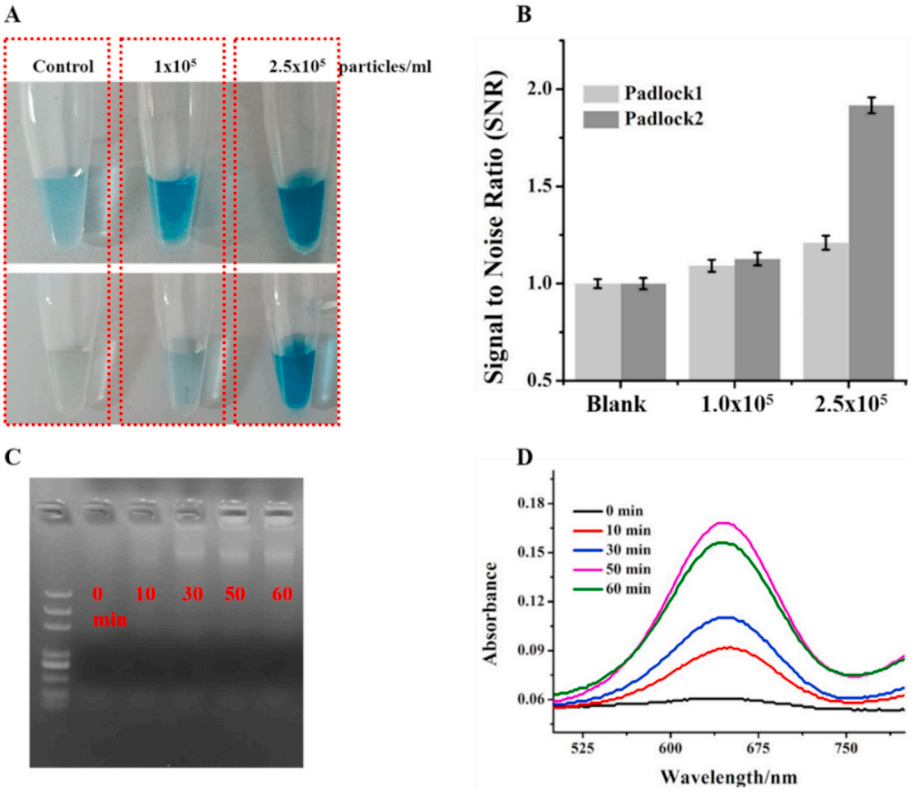


Fig. 4. Optimization of the experimental conditions. (A) The color intensity of exosomes detection induced by different padlock. (B) The signal to noise ratio of color intensity corresponding to Fig. 4A, Mean ± S. D., n = 3. (C) Agarose gel electrophoresis of RCA products with different reaction time. (D) UV–vis absorption spectrum of color signal with different RCA reaction time. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Au-HS) or primary amine groups (electrostatic) of HRP [29]. Thus, we firstly examined the size and homogeneity of GNPs through TEM and UV–vis absorption spectra. As shown in Fig. 2A, the GNPs possess uniform spherical morphology with an average diameter of 18 nm, and the surface of GNPs-HRP showed a halo indicating the successful modification of HRP. In addition, the red shift of UV–vis absorption spectra (Fig. 2B) and the different migration rates of GNPs in agarose electrophoresis (Fig. 2C) also showed the similar results. After the modification of GNPs with HRP, the GNPs-HRP could obtain the catalytic ability to TMB. Thus, TMB and H_2O_2 were further used to verify the catalytic activity of GNPs-HRP complex. As shown in Fig. 2D, the obvious color change can be observed in GNPs-HRP group and GNPs group showed negligible color change, suggesting the successful functionalization of HRP on the GNPs.

3.3. Feasibility verification of the biosensor for exosomes detection

To test the feasibility of our method, the probes used in this biosensor were firstly tested by native polyacrylamide gel electrophoresis (PAGE). As shown in Fig. 3A, the complex of trigger-aptamer was stable and could not be separated by padlock probe. Simultaneously, the analysis of RCA product also suggested padlock probe could not separate the trigger-aptamer complex (figure S1). Thus, we used the HL-60 cells derived exosomes to further verify the feasibility of the detection principle. According to the working principle, the trigger probe only can be released in the presence of exosomes, and then triggering the following RCA to produce color change based on the binding of GNPs-HRP. Therefore, we tested color change of TMB under the different condition. As shown in Fig. 3B, there is no color signal in the absence of exosomes (black curve), and the obvious color signal can be obtained in the presence of exosomes. In addition, the color change in the GNPs-HRP

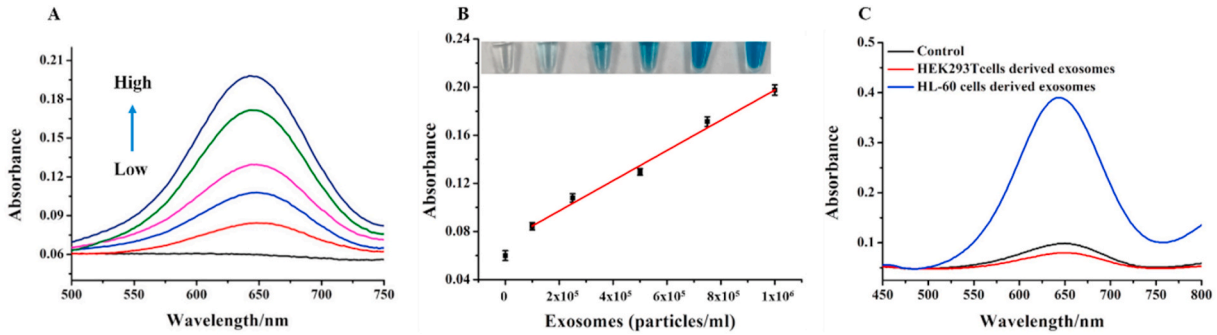


Fig. 5. (A) UV–vis absorption spectrum in the presence of different exosomes concentrations. (B) Standard curve of the UV–vis absorbance value versus the concentration of exosomes (Insert is the photo of color intensity with different exosomes concentrations), Mean ± S.D., n = 3. (C) The specificity of this method for leukemia cells derived exosomes detection. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Comparison of the LOD of current methods for exosomes detection.

No.	Method	LOD (particles/μL)	Ref.
1	Surface plasmon resonance	1×10^4	[22]
2	Electrochemical method	149	[24]
3	Fluorescence method	400	[25]
4	Colorimetric method	2.2×10^3	[26]
5	Colorimetric method	160	[27]
6	Colorimetric method	340	[28]
7	Colorimetric method	100	This work

group (blue curve) was more strongly than the SA-HRP (streptavidin-HRP) group (red curve), indicating the successful signal amplification by using GNPs-HRP.

3.4. Optimization of experimental conditions

To obtain the best sensing performance, the key experimental conditions, including the sequences of padlock and RCA reaction time, were optimized. Firstly, the sequences of padlock probe with different complementary base to trigger probe was optimized to reduce the background signal. As shown in Fig. 4A, the padlock probe 2 demonstrated lower background signal than padlock probe 1, indicating more complementary base between padlock probe and trigger probe is unfavorable to high signal noise ratio. So, we chose padlock probe 2 to induce RCA reaction. Next, we explored the effect of RCA time to the final detection signal, and find that the color intensity increased to the maximum when the RCA time was 50 min (Fig. 4D). The result of agarose electrophoresis also suggested the RCA products have reached a plateau at 50 min (Fig. 4C). Thus, the optimized RCA time of 50 min was used for the exosome detection in subsequent assays.

3.5. The detection performance of the method

Under the optimal experimental conditions, the capability of this method for quantitative exosomes analysis was evaluated. In the presence of exosomes derived from HL-60 cells, the color intensity of solution gradually increases with the increasing exosomes (Fig. 5A). The calibration curve ($y = 0.012x + 0.072$, $R^2 = 0.9944$) with good linear relationship could also be obtained between color signals of TMB against the concentration of exosomes (Fig. 5B), and the limit of detection (LOD) of this biosensor in PBS solution was calculated to be 100 particles/μL based on the 3σ method (σ was the standard deviation of color intensity for 10 blank samples). Compared to previous reported methods, this biosensor had a lower LOD (Table 1). In addition, the agarose gel electrophoresis of RCA product also increased with the increasing exosomes (figure S2).

The specificity was also utilized to assess the performance of this biosensor. Thus, HEK293T cells derived exosomes with no nucleolin expressed were used as control to explore the capability of this method in discriminating the exosomes derived from HL-60 cells. As shown in Fig. 5C, the color signals of HL-60 cells derived exosomes was significantly higher than HEK293T cells derived exosomes, indicating the proposed biosensor had an excellent selectivity for HL-60 cells derived exosomes.

3.6. The detection performance of biosensor for complex biological samples

To validate the clinical applicability of the designed biosensor, the anti-interference ability of this method for the exosomes detection in FBS was firstly investigated. As shown in Fig. 6A, the color intensity of exosomes spiked in 80% FBS showed no significant difference compared with that in PBS under the same concentration. Thus, we further evaluated the performance of our proposed method in clinical serum samples. The plasma samples from five leukemia patients and five healthy individuals were firstly collected and centrifuged to remove the cells and large vesicles. Then, 40 μl clinical samples were added into the detection system according to the protocol. The results showed that the signals could be directly observed by naked eye visualization (Fig. 6B), and successfully distinguished the leukemia patients with healthy people ($p < 0.01$), indicating this method possess promising potential for clinical application by sensing the nucleolin on the exosomes.

4. Conclusion

In conclusion, we constructed a colorimetric biosensor based on RCA induced dual signal amplification platform for visual analysis of leukemia-derived exosomes. Due to the using of aptamer, magnetic beads and dual signal amplification strategy, this method demonstrated high sensitivity (LOD, 100 particles/μL), good linearity ($R^2 = 0.9944$) and selectivity, low signal noise ratio and small sample volume (40 μL). Importantly, this biosensor could acquire the result through the naked eye visualization, without using large and complex instruments. Additionally, it could successfully distinguish the health and leukemia patients through the observation of color change, indicating a promising clinical application of this method in liquid biopsy of leukemia.

Author contributions

Chao Li: Conceptualization, Methodology, Software, Validation, Investigation, Writing- Reviewing and Editing, **Mengyang Zhou**: Validation, Formal analysis, Data curation. **Haoyu Wang**: Software, Visualization. **Jie Wang**: Formal analysis, Resources. **Lin Huang**: Conceptualization, Methodology, Writing – original draft, Project administration, Funding acquisition.

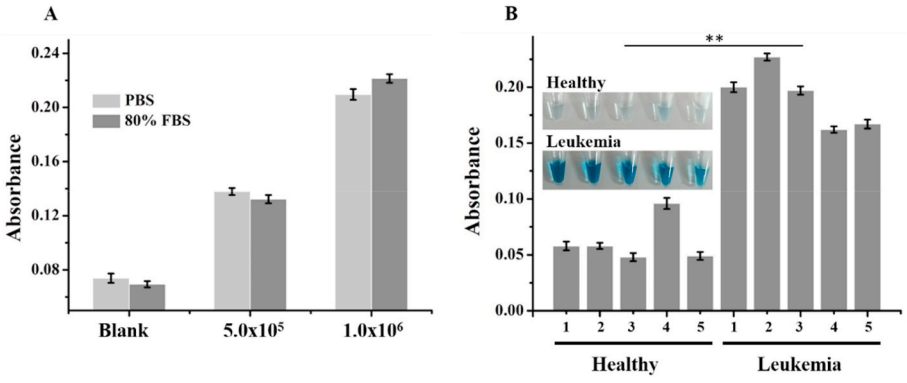


Fig. 6. (A) The color siganl of this biosensor after incubation with spiked exosomes in PBS and in 80% FBS. (B) The determination of exosomes in the plasma samples from healthy donors and leukemia patients (n = 5). Insert picture is the color intensity induced by exosomes of clinical samples. All data are mean ± standard variation (n = 3). Significance was tested by a two-tailed Student's t-test, **p < 0.01. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant number 32001006), the Scientific Research of BSKY from Anhui Medical University (XJ201930), and the Research Fund of Anhui Medical University (2019xkj014). The authors gratefully acknowledge the help of Mengfei Ren (Center for Scientific Research of College of Life Science, Anhui Medical University), and Haijian Cai (Center for Scientific Research of Anhui Medical University) for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2022.123444.

References

[1] M.E. Percival, C. Lai, E. Estey, C.S. Hourigan, Bone marrow evaluation for diagnosis and monitoring of acute myeloid leukemia, Blood Rev. 31 (2017) 185–192.
[2] L.S. Pessoa, M. Heringer, V.P. Ferrer, ctDNA as a cancer biomarker: a broad overview, Crit. Rev. Oncol. Hematol. 155 (2020) 103109.
[3] A. Vasseur, N. Kiavue, F.C. Bidard, J.Y. Pierga, L. Cabel, Clinical utility of circulating tumor cells: an update, Mol. Oncol. 15 (2021) 1647–1666.
[4] M.L. Cheng, E. Pectasides, G.J. Hanna, H.A. Parsons, A.D. Choudhury, G.R. Oxnard, Circulating tumor DNA in advanced solid tumors: clinical relevance and future directions, CA Cancer J. Clin. 71 (2021) 176–190.
[5] W. Yu, J. Hurley, D. Roberts, S.K. Chakrabortty, D. Enderle, M. Noerholm, X. O. Breakefield, J.K. Skog, Exosome-based liquid biopsies in cancer: opportunities and challenges, Ann. Oncol. 32 (2021) 466–477.
[6] S. Wang, K. Zhang, S. Tan, J. Xin, Q. Yuan, H. Xu, X. Xu, Q. Liang, D.C. Christiani, M. Wang, L. Liu, M. Du, Circular RNAs in body fluids as cancer biomarkers: the new frontier of liquid biopsies, Mol. Cancer 20 (2021) 13.
[7] F. Teng, M. Fussenegger, Shedding light on extracellular vesicle biogenesis and bioengineering, Adv. Sci. 8 (2020) 2003505.
[8] R. Kalluri, V.S. LeBleu, The biology, function, and biomedical applications of exosomes, Science 367 (2020) 6977.
[9] U. Erb, M. Zöller, Progress and potential of exosome analysis for early pancreatic cancer detection, Expert Rev. Mol. Diagn 16 (2016) 757–767.
[10] D. Yang, W. Zhang, H. Zhang, F. Zhang, L. Chen, L. Ma, L.M. Larcher, S. Chen, N. Liu, Q. Zhao, P. Tran, C. Chen, R.N. Veedu, T. Wang, Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics, Theranostics 10 (2020) 3684–3707.
[11] Y.H. Soung, S. Ford, V. Zhang, J. Chung, Exosomes in cancer diagnostics, Cancers 9 (2017) 8.
[12] A. Li, T. Zhang, M. Zheng, Y. Liu, Z. Chen, Exosomal proteins as potential markers of tumor diagnosis, J. Hematol. Oncol. 10 (2017) 175.
[13] Y. Wu, Y. Zhang, X. Zhang, S. Luo, X. Yan, Y. Qiu, L. Zheng, L. Li, Research advances for exosomal miRNAs detection in biosensing: from the massive study to the individual study, Biosens. Bioelectron. 177 (2020) 112962.
[14] F. Momen-Heravi, S.J. Gettingc, S.A. Moschos, Extracellular vesicles and their nucleic acids for biomarker discovery, Pharmacol. Ther. 192 (201) 170-187.
[15] Q. Zhu, L. Huang, Q. Yang, Z. Ao, R. Yang, J. Krzesniak, D. Lou, L. Hu, X. Dai, F. Guo, F. Liu, Metabolomic analysis of exosomal-markers in esophageal squamous cell carcinoma, Nanoscale 13 (2021) 16457–16464.
[16] Y. Yu, W.S. Zhang, Y. Guo, H. Peng, M. Zhu, D. Miao, G. Su, Engineering of exosome-triggered enzyme-powered DNA motors for highly sensitive fluorescence detection of tumor-derived exosomes, Biosens. Bioelectron. 167 (2020) 112482.
[17] L. Wang, Y. Deng, J. Wei, Y. Huang, Z. Wang, G. Li, Spherical nucleic acids-based cascade signal amplification for highly sensitive detection of exosomes, Biosens. Bioelectron. 191 (2021) 113465.
[18] L. Xu, R. Chopdat, D. Li, K.T. Al-Jamal, Development of a simple, sensitive and selective colorimetric aptasensor for the detection of cancer-derived exosomes, Biosens. Bioelectron. 169 (2020) 112576.
[19] J. Qian, Q. Zhang, M. Liu, Y. Wang, M. Lu, A portable system for isothermal amplification and detection of exosomal microRNAs, Biosens. Bioelectron. 196 (2022) 113707.
[20] S. Gandham, X. Su, J. Wood, et al., Technologies and standardization in Research on extracellular vesicles, Trends Biotechnol. 38 (2020) 1066–1098.
[21] Z. Yu, S. Lin, F. Xia, Y. Liu, D. Zhang, F. Wang, Y. Wang, Q. Li, J. Niu, C. Cao, D. Cui, N. Sheng, J. Ren, Z. Wang, D. Chen, ExoSD chips for high-purity immunomagnetic separation and high-sensitivity detection of gastric cancer cell-derived exosomes, Biosens. Bioelectron. 194 (2021) 113594.
[22] Y. Fan, X. Duan, M. Zhao, X. Wei, J. Wu, W. Chen, P. Liu, W. Cheng, Q. Cheng, S. Ding, High-sensitive and multiplex biosensing assay of NSCLC-derived exosomes via different recognition sites based on SPRi array, Biosens. Bioelectron. 154 (2020) 112066.
[23] Y. Wang, Q. Li, H. Shi, K. Tang, L. Qiao, G. Yu, C. Ding, S. Yu, Microfluidic Raman biochip detection of exosomes: a promising tool for prostate cancer diagnosis, Lab Chip 20 (2020) 4632–4637.
[24] X. Zhu, Z. Liu, J. Li, Z. Li, F. Si, H. Yang, J. Kong, Dual signal amplification based on polysaccharide-initiated ring-opening polymerization and click polymerization for exosomes detection, Talanta 233 (2021) 122531.
[25] Y.M. Xiong, L.L. He, M. Yang, Y. Wang, X.L. Liu, S.S. Ma, B. Yang, F.X. Guan, Proximity hybridization-mediated fluorescence resonance energy transfer for highly specific detection of tumor-derived exosomes: combining multiple exosomal surface markers, Sens. Actuators, B 353 (2022) 131126.
[26] F. He, H. Liu, X. Guo, B.C. Yin, B.C. Ye, Direct exosome quantification via bivalent-cholesterol-labeled DNA anchor for signal amplification, Anal. Chem. 23 (2017) 12968–12975.
[27] Y. Zhang, D. Wang, S. Yue, Y. Lu, C. Yang, J. Fang, Z. Xu, Sensitive multicolor visual detection of exosomes via dual signal amplification strategy of enzyme-catalyzed metallization of Au nanorods and hybridization chain reaction, ACS Sens. 4 (2019) 3210–3218.
[28] Y. Xia, T. Chen, G. Chen, Y. Weng, L. Zeng, Y. Liao, W. Chen, J. Lan, J. Zhang, J. A. Chen, nature-inspired colorimetric and fluorescent dual-modal biosensor for exosomes detection, Talanta 214 (2020) 120851.
[29] R. Cui, H. Huang, Z. Yin, D. Gao, J.J. Zhu, Horseradish peroxidase-functionalized gold nanoparticle label for amplified immunoanalysis based on gold nanoparticles/carbon nanotubes hybrids modified biosensor, Biosens. Bioelectron. 23 (2008) 1666–1673.