Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy xxx (xxxx) xxx

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters

Neda Fakhri [a], Shima Abarghoei [b], Mehdi Dadmehr [c], Morteza Hosseini [b, *], Hossein Sabahi [b], Mohammad Reza Ganjali [d, e]

[a] School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[b] Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran
[c] Department of Biology, Payame Noor University, Tehran, Iran
[d] Center of Excellence in Electrochemistry, School of Chemistry, College of Science, University of Tehran, Tehran, Iran
[e] Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 20 May 2019
Received in revised form
26 July 2019
Accepted 12 September 2019
Available online xxx

Keywords:
Paper based
Colorimetric
DNA-Ag/Pt nanoclusters
Peroxidase
Cancer

ABSTRACT

Abnormal expression of MicroRNA-21 (miRNA-21) is considered to be a reliable biomarker for the early diagnosis of cancer. In this work, a novel paper based biosensor was fabricated to detect sub-micro molar concentrations of miRNA-21 based on peroxidase mimetic activity of DNA-templated Ag/Pt nanoclusters (DNA-Ag/Pt NCs), which could catalyze the reaction of hydrogen peroxide and 3,3′,5,5′ tetramethylbenzidine (TMB), to produce a blue color. The Mechanism of reaction was based on the inhibition effect of miRNA-21 on peroxidase-like activity of nanosensor which resulted to quantitative determination of miRNA-21 concentration. It was found that miRNA-21 could be linearly detected in the range from 1-700 pM ($A_{652} = 0.16x-0.96$, $R^2 = 0.99$; x = -log [miRNA-21]) with a detection limit of 0.6 pM. Moreover, a paper assay was carried out on a Y-shaped paper-based microfluidic device in order to use the distinctive features of micro-channels such as short response time, very low reagent volume, low fabrication cost, etc. After performing paper based assay, a good linear range was observed between 10-1000 pM ($y = 0.06x+147.48$, $R^2 = 0.99$; x = [miRNA-21]) with detection limit of 4.1 pM. The practical application of proposed method for detection of miRNA-21 in real sample was assayed in the human urine sample and indicated the colorimetric method had acceptable accuracy.

1. Introduction

MicroRNAs (miRNAs) are a class of 19–23 nucleotide single stranded noncoding RNA molecules involved in regulation of gene expression and control the diverse physiological processes including cell differentiation, proliferation, immunity, and apoptosis. It has been reported that abnormal expression of specific miRNAs is closely related to various diseases, especially different types of cancer. miRNA-21 is an example that found to be over expressed in many cancer tissues, such as gastric, breast, lung, colorectal, and prostate cancers. Thus, quantitative assay of miRNA-21 can be employed as biomarkers for early diagnosis of cancer [1,2].

The specific features of miRNA-21, such as short length, high sequence homology among family members, and low abundance in total RNA samples, make it difficult to detect them. However, the arrival of biosensing methods, such as electrochemical biosensors, luminescence sensors and colorimetric sensors, has led to the introduction of a variety of powerful methods for accurate and quantitative detection of miRNA-21 expression [3–5]. Among them, colorimetric sensors have recently received great attention because of their very simple fabrication and application, low cost, and visual detection in homogeneous solutions without further separation and washing steps [6,7]. However, it is still a challenge to develop colorimetric assays with such high sensitivity as electrochemical methods. Recently, many metalic nanoparticles and nanoclusters with enzyme mimetic behavior have been reported for detection of various analytes such as heavy metal ions, DNA and proteins [8–13].

Metal nanoclusters consist of several metal atoms, with sub-nanometer-size cores and discrete energy levels, which are regarded as an interface between metal atoms and particles. These structural features give them unique chemical and optical properties. They have also advantages such as easy preparation, low

* Corresponding author.
E-mail address: smhosseini@khayam.ut.ac.ir (M. Hosseini).

https://doi.org/10.1016/j.saa.2019.117529
1386-1425/

toxicity and excellent biocompatibility and photo-stability [14–18].

In spite of many conducted studies to explore DNA-Ag NCs and DNA-Au NCs as fluorescent probes, their application in enzyme mimetic catalysis is seldom reported because of their poor catalytic characteristics. Recently, DNA-Ag/Pt NCs were reported to have much stronger catalytic activity than DNA-Pt NCs and DNA-Ag NCs due to the appropriate composition of Ag/Pt NCs which played a key role in their catalytic activity [19,20]. Nonetheless, DNA-Ag/Pt NCs have not been explored to detect miRNA-21 so far.

With the advent of microfluidic chips which harnesses the benefits of exquisite micro scale fluid physics a new era of sensing has begun in which microfluidic devices reduce the sample and reagent use, reduce the size and cost of tests, and improve overall automation [9]. In 2007, Whitesides et al proposed the potential of paper as a promising material for fabricating microfluidic paper-based analytical devices (μPADs) [21]. So far, a number of processing techniques has been introduced to construct μPADs by patterning hydrophilic/hydrophobic microstructures on paper substrates, including paper cutting [22], Wax printing [23], wax dipping [24], plotting [25], inkjet printing [26], plasma treatment [27], and photolithography [28]. Compared to conventional microfluidic chips made of glass and polymer substrates, μPADs possess many unique advantages including, low-cost, ease of fabrication, strong capillary action and good biological compatibility for applications in clinical diagnosis, food quality control and environmental monitoring [22].

In the present study, we reported one-step approach for DNA template synthesis of Ag/Pt bimetallic nanoclusters through facile method. The synthesized DNA—Ag/Pt NCs showed peroxidase-like catalytic activity and could catalyze hydrogen peroxide (H_2O_2)-mediated oxidation of the substrate, 3,3′,5,5′tetramethylbenzidine (TMB) and produce a blue color solution. Following we found that miRNA-21 can inhibit the peroxidase-like activity of DNA-Ag/Pt NCs selectively. Based on these results, the DNA-Ag/Pt NCs were utilized to develop a sensitive and selective detection method for miRNA-21. To the best of our knowledge, it is the first report to explore a colorimetric detection for miRNA-21 based on the peroxidase-like activity of the DNA-Ag/Pt NCs (Scheme 1).

2. Materials and methods

Sodium borohydride ($NaBH_4$) and Silver nitrate ($AgNO_3$), were purchased from Merck. K_2PtCl_4 and 3, 3′, 5, 5′-tetramethylbenzidine (TMB) were prepared from Sigma Aldrich. The following oligonucleotides were synthesized by Shanghai Generay Biotech Co. The oligonucleotides stocks were prepared with TE buffer (containing 1 M Tris-HCl and 0.5 mL EDTA, pH 7.5). All chemicals were of analytical grade and used without further purification. Other reagents such as hydrogen peroxide were also purchased from Merck. The water used in all experiments was ultrapure water.

Probe: 5′- CCCCCCCC TCAACATCAGTCTGATAAGCTA-3′.

Target: 5′- UAGCUUAUCAGACUGAUGUUGA-3′.

Non complementary target: 5′- CGAATTTTTGGAGGAAGGAGG-3′.

One base mismatches target: 5′- UAGCUUCUCAGACUGAU-GUUGA-3′.

2.1. Apparatus

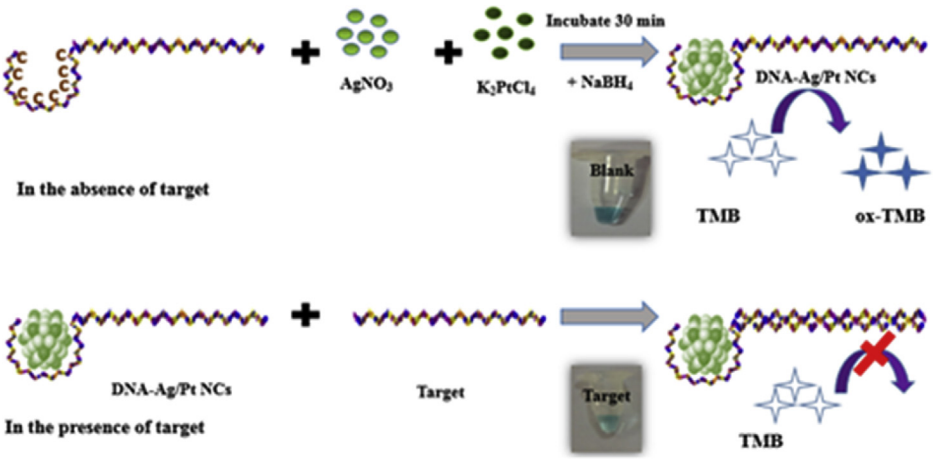
A spectrometer (PerkinElmer lambda25) was used to record the ultraviolet-visible (UV—vis) spectra of theTMB-H_2O_2solution. KYKY-EM3200 Transmission Electron Microscopy (TEM) was used to determine the size and morphology of synthesized NCs. Energy dispersive X-ray (EDX) was recorded on MIRA III field emission scanning electron microscope (TESCAN, Czech Republic).

2.2. Preparation of DNA-Ag/Pt NCs

DNA-Ag/Pt NCs was synthetized according to previously published [19,20]. In this work we chose a C-rich ssDNA (single stranded DNA) template sequence, 5′-CCCCCCCCTCAA-CATCAGTCTGATAAGCTA-3′, for direct synthesis of Ag NCs. Briefly, $AgNO_3$(8 μL, 8 mM), K_2PtCl_4 (9 μL, 2 mM), and the ssDNA (3 μL, 100 μM) in 30 μL of phosphate buffer (20.0 mM, pH7.4) were mixed thoroughly. then, 8 μL of NaBH4 (8 mM) which was previously prepared freshly was added to the solution under vigorous stirring. Afterwards, the as prepared solution was incubated at 37 °C for 30 min in the dark. Finally, the mixture was incubated for 3 h in the dark at room temperature. The obtained DNA- Ag/Pt NCs with the concentration of 5.1 μM was stored at 4 °C for further use.

2.3. Detection of miRNA-21

A colorimetric assay for miRNA-21 was carried out as follows. First, different concentrations of miRNA-21 (ranging from 1 pM-700pM) were added to 58 μL of as-prepared DNA-Ag/Pt NCs solution. Next, the mixed solution was incubated at 37 °C for 30 min. Then, 10 μL of TMB (10 mM) and 10 μL of H_2O_2 (10 mM) were added respectively and finally, the resultant solution was used for both adsorption spectroscopy measurement at 652 nm (A652) and paper test analysis.



Scheme 1. Schematic illustration of synthesis of DNA-Ag/Pt NCs along with its sensing procedure.

Please cite this article as: N. Fakhri et al., Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, https://doi.org/10.1016/j.saa.2019.117529

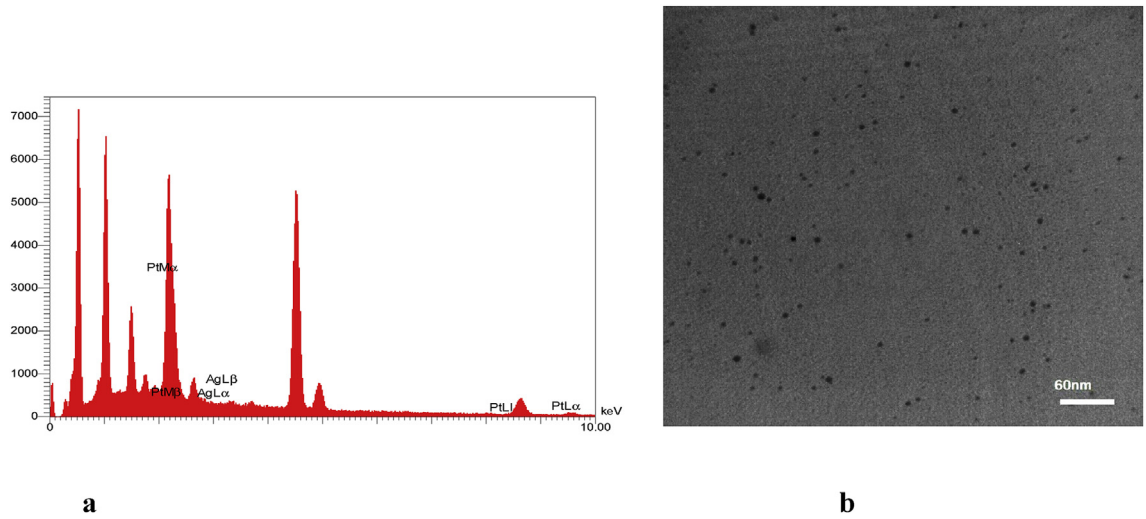


Fig. 1. (a)EDX analysis of DNA-Ag/Pt NCs, (b)TEM image of DNA-Ag/Pt NCs.

2.4. Fabrication of paper-based microfluidic device

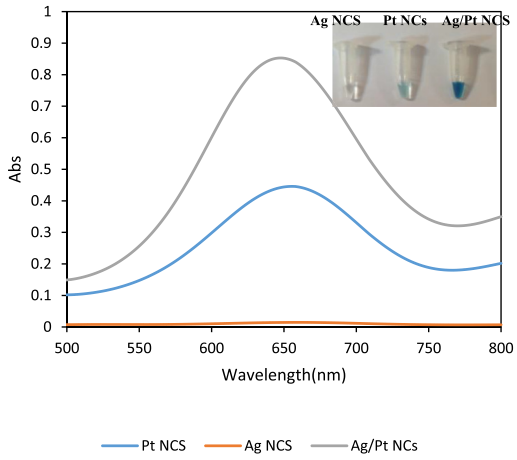
It has been reported that in paper based microfluidic devices the ability to control the flow of liquids, including direction and time, is very important in creating more powerfully analytical devices on paper. A typical method to control liquid flow speed is to change the hydrophilic channels length and width. However, there is an upper limit to the size of a μPAD, especially when volumes of sample are limited. In this work, cutting method was used to change geometry of paper and then adjusting the flow speed by capillary forces [22]. The Whatman filter No.1 was cutted by a CO2 laser cutter/engraver machine. The generated heat by the laser burned the paper and cut it in the desired pattern which forms the hydrophobic barriers needed to form microchannel. A Y-shaped two step microchannel was designed in which the widths of channels were designed to be 600 μm. However, after applying laser, the width of channel became less than the designed pattern due to the burning effect of laser. In order to prevent the interfering effects of impurities caused by burning, papers were soaked in distilled water for 1 day after being cut. Then, PVC sheets were used in order to prevent the leakage of reagents from the bottom of the sensing device channels.

2.5. Paper assay

To conduct the paper assay, 9 μL of DNA-Ag/Pt NCs was spotted on the middle spot zone followed by addition of 3 μL of sample containing miRNA-21 to the right spot zone. In the upper spot zones, 3 μL of TMB (10 mM) was casted and left to dry at room temperature. Finally, 3 μL of 10 mM H_2O_2 was injected from the bottom entrance. The color of the upper left side spot zone turned blue as soon as the mixture reached that area while the left spot zone remained colorless due to the interference effect of miRNA-21. The color change could be distinguished by naked eye except for very low concentrations. To overcome this difficulty in distinguishing the color change, the intensity of the grey value was determined by Image J software for quantitative analysis.

2.6. Analysis of real samples

Previously described method was carried out for human blood plasma samples collected from a local hospital. Different concentrations of miRNA-21 (1, 10, 100 and 500 pM) were spiked in the samples and vortexed for 2 min at room temperature. After diluting by ultra-pure water (10-fold), DNA-Ag/Pt NCs were added to the samples for the detection of miRNA-21 in the presence of interfering molecules. In the end, in order to assess the peroxidase activity, TMB and H_2O_2were added to the as-prepared solution.



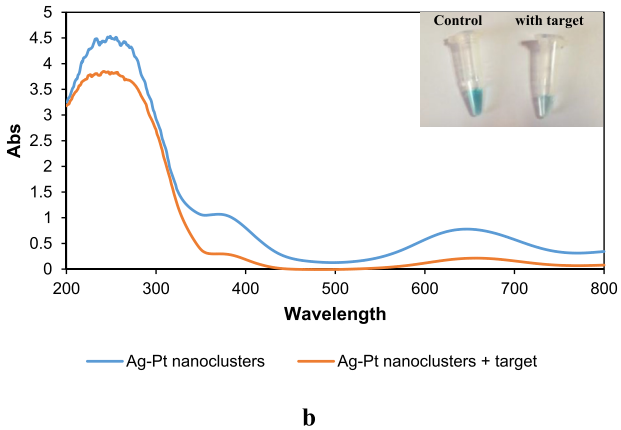


Fig. 2. (a) Comparison of catalytic activity of DNA-Ag NCs, DNA-Pt NCs, and DNA-Ag/Pt NCs; inset picture depicts the color change of the aforementioned NCs, (b)Absorbance spectra of DNA-Ag/Pt NCs before and after the addition of miRNA-21, inset image illustrates the color change of the proposed nanosensor.

Please cite this article as: N. Fakhri et al., Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, https://doi.org/10.1016/j.saa.2019.117529

3. Results and discussion

3.1. Characterization of synthesized DNA-Ag/Pt NCs

For determining the elemental composition of the as synthesized DNA-Ag/Pt NCs, Energy-dispersive X-ray(EDX) was conducted. As shown in Fig. 1a, successful formation of bimetallic NCs can be clearly detected by elemental Ag and Pt spectrum. In addition, using transmission electron microscopy (TEM) it was found that DNA- Ag/Pt NCs are about 4 nm in size and roughly spherical (Fig. 1b).

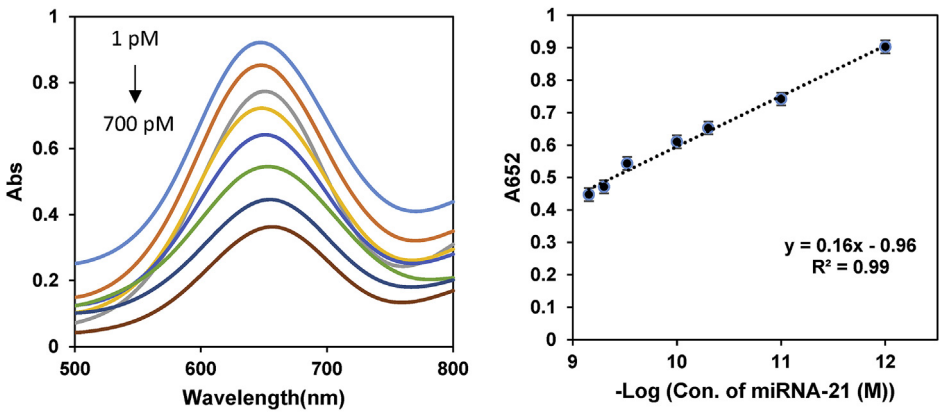
To examine the peroxidase-like activity of the synthesized DNA-Ag/Pt NCs and compare to other nanoclusters like DNA-Ag NCs, DNA-Pt NCs, the catalytic oxidation of the typical peroxidase substrate TMB in the presence of H_2O_2 was carried out. Color changes of different nanoclusters can be observed from Fig. 2. As it is shown in Fig. 2, the DNA-Ag/Pt NCs showed a significant peroxidase-like activity compare to DNA-Ag/NCs and DNA-Pt/NCs. The TMB + H_2O_2+ DNA-Ag/Pt NCs system distinguished by obvious absorption peak at 652 nm, which indicated DNA-Ag/Pt NCs could act as a peroxidase to oxidase TMB to produce a blue color in the presence of H_2O_2. This higher peroxidase-like activity of Ag/Pt NCs could be due to the electronic charge transfer effect and the synergistic effect among Ag and Pt that could promote the adsorption and the efficient oxidation of reactants, thereby offering an improved catalytic activity [19,20].

3.2. The inhibition effect of miRNA-21 on peroxidase like activity of DNA-Ag/Pt NCs

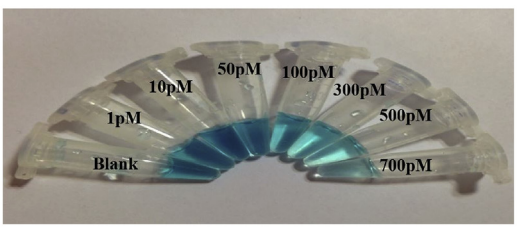
Fig. 2b illustrated that the oxidation of colorless TMB by H_2O_2 was catalyzed by Ag/Pt NCs to make the solution blue as a result of the oxidation product. This blue color of the solution led to a strong absorbance peak around 652 nm. However, when 100 μM of miRNA-21 was added to the reaction, it was inhibited oxidation of TMB significantly and the absorbance peak at 652 nm reached to only 27% of that in the absence of miRNA-21. These results confirmed that miRNA-21 can lead to the inhibition of DNA-Ag/Pt NCs peroxidase-like activity. In this DNA-Ag/Pt NCs sensing system, NCs can be protected by DNA template molecules to some extent. Nevertheless, we deduced that there is a strong interaction between miRNA-21 and the DNA-Ag/Pt NCs which lead to the inhibition of color change.

3.3. Optimization conditions

According to the above mentioned characteristics of DNA- Ag/Pt NCs can be used for colorimetric detection of miRNA-21. In order to get the best sensing performance in terms of sensitivity, different catalytic reaction conditions such as concentration of H_2O_2 and TMB, time, and pH of PBS buffer was assessed. It was found that the largest absorbance (the A652 value of the solution without the addition of miRNA-21 subtracted from that with the addition of 700pM miRNA-21 can be obtained when incubation time of the DNA-Ag/Pt NCs and miRNA-21 was set at 30 min (Fig. S1). When the catalytic reaction was kept to continue for a longer time, the absorbance diminished slightly probably because of the reduced DNA-Ag/Pt NCs stability. Among PBS and NaAc buffer, it was found that PBS can be more effective in regulating the pH change in the system. Moreover, the optimization of pH in the range of 2–9 was done and it was found that a pH value of 7 could lead to the best color change in the solution (Fig. S2). Furthermore, the optimization of both H_2O_2 and TMB was carried out and it was observed that both of them, with the same concentration of 0.01 M, can lead to the best responding signal (Figs. S3 and S4).



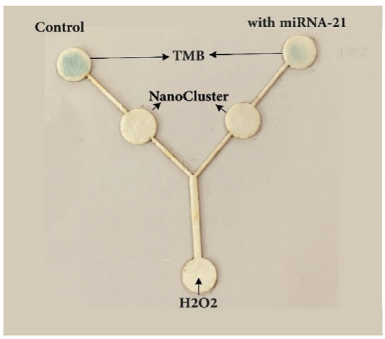
(a) (b)



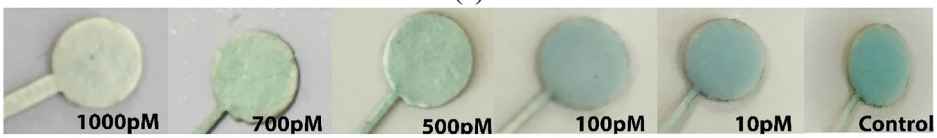
(c)

Fig. 3. (a) Absorbance spectra of catalytic activity of DNA-Ag/PtNCs with TMB and H_2O_2 in the presence of different concentrations of miRNA-21, (b) The peak absorbance change of 652 nm is linear with miRNA-21 concentrations (c) Incremental color change observed with naked eye for nanosensor with increasing concentration of miRNA-21 (1–700pM).

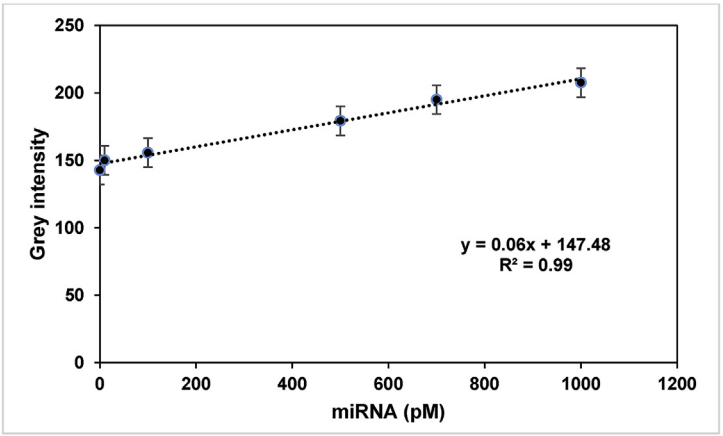
Please cite this article as: N. Fakhri et al., Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, https://doi.org/10.1016/j.saa.2019.117529



(a)



(b)



(c)

Fig. 4. Analytical results of the paper-based device; (a) Paper based microfluidic platform used for the detection of 1000pM miRNA-21 solution. (b) Different colors related to different concentrations of miRNA-21 over the range of 1 pM–700 pM (c) Detection on the paper-based platform linearly changes from 1 pM to 700 pM of miRNA-21 concentration.

Table 1
Comparison of different miRNA-21 sensing systems reported in litretures

Detection method	Material/method used	Linear range	LOD	Ref
Colorimetric	T7 exonuclease	10pM-100 nM	0.69pM	[29]
Colorimitric	Graphene/Au-NPs	10nM-0.98 μM	0.0032 μM	[30]
Fluorescent	Silver Nanocluster	0.2 nM-30nM	0.1 nM	[31]
SPR	GO/AuNPs hybrids amplification	–	1 fM	[32]
Electrochemical	Au NP	5.0 fM–1.0 pM	1.6 fM	[33]
Fluorescent	DNA-Ag NCs	0–100 nM	0.2 nM	[1]
Dual-Wavelength ECL Ratiometry	Au Nanoparticles $Ru(bpy)_3$/g-C_3N_4 Nanosheet	1.0 f. to 1.0 nM	0.5 fM	[34]
Colorimetric	DNA-Ag/Pt NCs	1.0–700pM	0.6pM	this work

3.4. Detection of miRNA-21 by the colorimetric assay

Under the optimum conditions, different concentrations of miRNA-21 were mixed with the DNA-Ag/Pt NCs and then the resultant solution was added into the reaction solution, containing TMB-H_2O_2, which resulted in decremented color change with increasing the miRNA-21 concentration in the solution (Fig. 3). As a result, the absorption peak of the reaction solution at 652 nm diminished gradually as concentration of miRNA-21 increased from 1 to 700 pM (Fig. 4a). As can be seen from Fig. 4b, it was observed that there is a linear relationship between miRNA-21 concentrations ranging from 1-700 pM and the A652 values ($A_{652} = 0.16x-0.96$; $R^2 = 0.99$). Furthermore, the limit of detection of this sensor was estimated be to 0.6 pM as a signal-to-noise ratio of 3.

To assess the performance of the proposed sensor, the properties of this method was compared with the previously reported

Please cite this article as: N. Fakhri et al., Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, https://doi.org/10.1016/j.saa.2019.117529

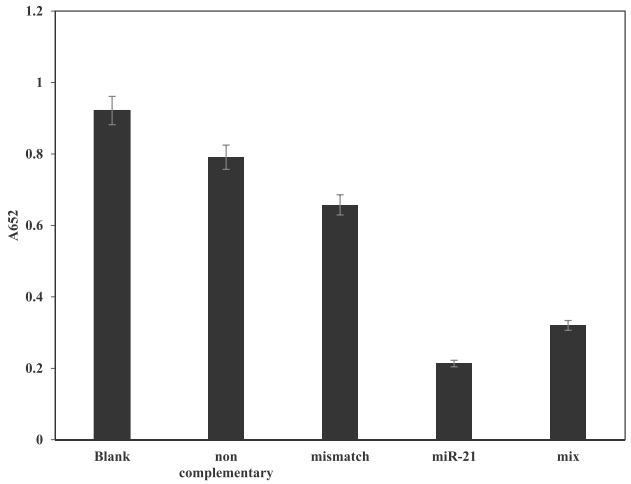


Fig. 5. Selectivity of the miRNA-21 biosensor toward a perfectly matched target RNA, one-base mismatched target RNA and noncomplementary target RNA at the same concentration (600pM) (a) color change, (b) The peak absorbance change of 652 nm toward aforementioned targets.

biosensors [29–34]. As can be seen (Table 1), the sensitivity of the analytical assay was comparable with those of other biosensors except for electrochemical based methods which had low limits of detection. However, the electrochemical based methods need such experimental steps such as labeling, immobilization, washing, and enzyme amplification, which makes them time consuming and costly. Nonetheless, in comparison with current sensors, the results in this work obviously showed an excellent sensitivity and selectivity toward miRNA-21 detection.

3.5. Paper-based colorimetric test

Paper-based analytical biosensors are regarded as promising tools with low cost, affordable and simple design benefits which required very low sample volume. Because of their advantages they are in highly considered especially in low income countries.

A simple Y shaped 2 step paper-based device was used for testing the proposed method which is depicted in Fig. 4 a. By the Increasing of the miRNA-21 concentration over the range of 10 pM–1000 pM, the color intensity changed which was obviously observable on paper platform similar to the liquid phase reports. A smartphone was used to capture photos of the μPAD. Following the obtained images were analyzed with Image J software to determine the related concentrations. The results showed that the grey intensity increased as the concentration of miRNA-21 increased in the solution (Fig. 4 b,c). According to results, the linear range of paper-based method for miRNA-21 was between 10 pM to 1000 pM with detection limit of 4.1 pM.

3.6. Selectivity of the DNA-Ag/Pt NCs based assay

To further investigate the potential of the proposed assay, selectivity test was conducted toward a perfectly matched target miRNA, one-base mismatched target miRNA, noncomplementary target RNA and mix miRNA at the same concentration (700 pM). As can be seen in Fig. 5, the absorbance of the DNA-Ag/Pt NCs toward the perfectly matched target miRNA was much stronger than the mismatched miRNA proving the high sensitivity of the proposed method toward miRNA-21 detection. Thus, this method showed sensitive and simple approach with high quantitative capacity for quantitative detection of miRNA-21 sequence, which can be applied in clinical applications for the diagnosis of miRNA-21.

Table 2
Analytical results for miRNA-21 in human serum samples using the proposed method *(n = 3)*.

Samples	Added (pM)	Founded (pM)	Recovery (%)	Color change
1	1	1.07	107.0 (±0.6)	
2	10.0	10.6	106.0 (±0.8)	
3	100.0	93.8	93.8 (±0.5)	
4	500.0	530.3	106.0 (±1.0)	

3.7. Analysis of real samples

To determine whether or not the proposed testing system is applicable in the real sample, the filtrated human blood plasma obtained from a local hospital was used. Different concentrations of miRNA-21 along with their complementary probes were added to the pretreated blood plasma. After 30 min of incubation at room temperature, TMB and H_2O_2 were introduced to the as prepared solution according to the previous section. Table 2 demonstrated that the obtained results confirmed the previous observations, and showed a recovery of 93.8–106.0%. It was also seen that the presence of molecules in our plasma samples had no significant interference with our assay system, proving that the proposed system can be practically applied to detect miRNA-21 in real samples.

4. Conclusion

In summary, a novel, simple, rapid and highly sensitive colorimetric method for the detection of miRNA-21 was introduced. The assay mechanism was based on inhibition effect of miRNA-21 on peroxidase-like activity of DNA-Ag/Pt NCs. To the best of our knowledge, the presented method is the first reported paper colorimetric miRNA-21 assay by nanocluster catalytic activity which resulted in a limit of detection of 0.6pM for miRNA-21 detection. The DNA-Ag/Pt NCs probe preparation including hybridization and reduction steps was facile, affordable, and easily accessible in several laboratories. Moreover, it was not required to additionally functionalize the DNA-templated nanoclusters and avoid complex operations. The introduced DNA-Ag/Pt NCs based biosensor demonstrated a promising and applicable response in quantitative detection of miRNA in real biological samples.

Acknowledgments

The authors thank the research Council of University of Tehran for financial support of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2019.117529.

References

[1] H. Kim, S. Kang, K.S. Park, H.G. Park, Enzyme-free and label-free miRNA detection based on target-triggered catalytic hairpin assembly and fluorescence enhancement of DNA-silver nanoclusters, Sens. Actuators, B 260 (2018) 140–145.
[2] H. Wu, Y. Liu, H. Wang, J. Wu, F. Zhu, P. Zou, Label-free and enzyme-free colorimetric detection of microRNA by catalyzed hairpin assembly coupled with hybridization chain reaction, Biosens. Bioelectron. 81 (2016) 303–308.
[3] S. Bi, Y. Cui, L. Li, Dumbbell probe-mediated cascade isothermal amplification: a novel strategy for label-free detection of microRNAs and its application to real sample assay, Anal. Chim. Acta 760 (2013) 69–74.
[4] Y.S. Borghei, M. Hosseini, An approach toward miRNA detection via different thermo-responsive aggregation/disaggregation of CdTe quantum dots, RSC Adv. 8 (2018) 30148–30154.
[5] M. Dadmehr, M. Hosseini, S. Hosseinkhani, M.R. Ganjali, R. Sheikhnejad, Label free colorimetric and fluorimetric direct detection of methylated DNA based on silver nanoclusters for cancer early diagnosis, Biosens. Bioelectron. 73 (2015) 108–113.
[6] Y.S. Borghei, M. Hosseini, M. Dadmehr, Hosseinkhani, M.R. Ganjali, R. Sheikhnejad, Visual detection of cancer cells by colorimetric aptasensor based on aggregation of gold nanoparticles induced by DNA hybridization, Anal. Chim. Acta 904 (2016) 92–97.
[7] D. Li, W. Cheng, Y. Yan, Y. Zhang, Y. Yin, H. Ju, S. Ding, A colorimetric biosensor for detection of attomolar microRNA with a functional nucleic acid-based amplification machine, Talanta 146 (2016) 470–476.
[8] Z. Wang, X. Yang, J. Yang, Y. Jiang, N. He, Peroxidase-like activity of mesoporous silica encapsulated Pt nanoparticle and its application in colorimetric immunoassay, Anal. Chim. Acta 862 (2015) 53–63.
[9] S. Abarghoei, N. Fakhri, Y.S. Borghei, M. Hosseini, M.R. Ganjali, A colorimetric paper sensor for citrate as biomarker for early stage detection of prostate cancer based on peroxidase-like activity of cysteine-capped gold nanoclusters, Spectrochim. Acta, Part A 210 (2019) 251–259.
[10] Z. Dehghani, M. Hosseini, J. Mohammadnejad, M.R. Ganjali, Novel colorimetric sensor based on peroxidase-like activity of chitosan-stabilized Au/Pt nanoclusters for trace lead, Anal. Methods 11 (2019) 684–690.
[11] Z. Dehghani, M. Hosseini, J. Mohammadnejad, B. Bakhshi, A.H. Rezayan, Colorimetric aptasensor for *Campylobacter jejuni* cells by exploiting the peroxidase like activity of Au@Pd nanoparticles, Microchim. Acta. 185 (2018) 448.
[12] M. Rahimi-Nasrabadi, F. Mizani, M. Hosseini, A.H. Keihan, M.R. Ganjali, Detection of hydrogen peroxide and glucose by using Tb2 (MoO4) 3 nanoplates as peroxidase mimics, Spectrochim. Acta 186 (2017) 82–88.
[13] M. Hosseini, M. Aghazadehb, M.R. Ganjali, A facile one-pot synthesis of cobalt-doped magnetite/graphene nanocomposite as peroxidase mimetics in dopamine detection, New J. Chem. 41 (2017) 12678–12684.
[14] Y.S. Borghei, M. Hosseini, M.R. Ganjali, H. Ju, Colorimetric and energy transfer based fluorometric turn-on method for determination of microRNA using silver nanoclusters and gold nanoparticles, Microchim. Acta 185 (2018) 286.
[15] Y.S. Borghei, M. Hosseini, M.R. Ganjali, S. Hosseinkhani, Label-free fluorescent detection of microRNA-155 based on synthesis of hairpin DNA-templated copper nanoclusters by etching (top-down approach), Sens. Actuators, B 248 (2017) 133–139. 21.
[16] M.I. Halawa, F. Wu, A. Nsabimana, B. Lou, G. Xu, Inositol directed facile "green" synthesis of fluorescent gold nanoclusters as selective and sensitive detecting probes of ferric ions, Sens. Actuators, B 257 (2018) 980–987.
[17] M.I. Halawa, F. Wu, T.H. Fereja, B. Lou, G. Xu, One-pot green synthesis of supramolecular Β-cyclodextrin functionalized gold nanoclusters and their application for highly selective and sensitive fluorescent detection of dopamine, Sens. Actuators, B 254 (2018) 1017–1024.
[18] M.I. Halawa, W. Gao, M. Saqib, S.A. Kitte, F. Wu, G. Xu, Sensitive detection of alkaline phosphatase by switching on gold nanoclusters fluorescence quenched by pyridoxal phosphate, Biosens. Bioelectron. 95 (2017) 8–14.
[19] C. Zheng, A.X. Zheng, B. Liu, X.L. Zhang, Y. He, J. Li, Cheng Zheng, A.X. Zheng, B. Liu, X.L. Zhang, Y. He, J. Li, H.H. Yang, G. Chen, One-pot synthesized DNA-templated Ag/Pt bimetallic nanoclusters as peroxidase mimics for colorimetric detection of thrombin, Chem. Commun. 50 (2014) 13103–13106. 22.
[20] H.A. Kermani, M. Hosseini, A. Miti, M. Dadmehr, G. Zuccheri, S. Hosseinkhani, M.R. Ganjali, A colorimetric assay of DNA methyltransferase activity based on peroxidase mimicking of DNA template Ag/Pt bimetallic nanoclusters, Anal. Bioanal. Chem. 410 (2018) 4943–4952.
[21] A.W. Martinez, S.T. Phillips, M.J. Butte, G.M. Whitesides, Patterned paper as a platform for inexpensive, low-volume, portable bioassays, Angew. Chem. Int. Ed. 46 (2007) 1318–1320.
[22] N. Fakhri, Morteza Hosseini, Omid Tavakoli, Aptamer-based colorimetric determination of Pb 2+ using a paper-based microfluidic platform, Analytical methods 10 (36) (2018) 4438–4444.
[23] E. Carrilho, A.W. Martinez, G.M. Whitesides, Understanding wax printing: a simple micropatterning process for paper-based microfluidics, Anal. Chem. 81 (2009) 7091–7095.
[24] T. Songjaroen, W. Dungchai, O. Chailapakul, W. Laiwattanapaisal, Novel, simple and low-cost alternative method for fabrication of paper-based microfluidics by wax dipping, Talanta 85 (2011) 2587–25933J.
[25] Nie, Y. Zhang, L. Lin, C. Zhou, S. Li, L. Zhang, J. Li, Lowcost fabrication of paper-based microfluidic devices by onestep plotting, Anal. Chem. 84 (2012) 6331–6335.
[26] K. Abe, K. Kotera, K. Suzuki, D. Citterio, Inkjet-printed paperfluidic immunechemical sensing device, Anal. Bioanal. Chem. 398 (2010) 885–893.
[27] C.L. Sones, I.N. Katis, P.J.W. He, B. Mills, M.F. Namiq, P. Shardlow, M. Ibsen, R.W. Eason, Laser-induced photo-polymerisation for creation of paper-based fluidic devices, Lab Chip 14 (2014) 4567–4574.
[28] L. OuYang, C. Wang, F. Du, T. Zheng, H. Liang, Electrochromatographic separations of multicomponent metal complexes on a microfluidic paper-based device with a simplified photolithography, RSC Adv. 4 (2014) 1093–1101.
[29] Y. Sang, Y. Xu, L. Xu, W. Cheng, X. Li, J. Wu, S. Ding, Colorimetric and visual determination of microRNA via cycling signal amplification using T7 exonuclease, Microchim. Acta 184 (2017) 2465–2471.
[30] H. Zhao, Y. Qu, F. Yuan, X. Quan, A visible and label-free colorimetric sensor for miRNA-21 detection based on peroxidase-like activity of graphene/gold-nanoparticle hybrids, Anal. Methods 8 (2016) 2005–2012.
[31] M. Hosseini, A. Akbari, M.R. Ganjali, M. Dadmehr, A.H. Rezayan, A novel label-free microRNA-155 detection on the basis of fluorescent silver nanoclusters, J. Fluoresc. 25 (2015) 925–929.
[32] Q. Wang, Q. Li, X. Yang, K. Wang, S. Du, H. Zhang, Y. Nie, Graphene oxide-gold nanoparticles hybrids-based surface plasmon resonance for sensitive detection of microRNA, Biosens. Bioelectron. 77 (2016) 1001–1007.
[33] X. Miao, W. Wang, T. Kang, J. Liu, K.K. Shiu, C.H. Leung, D.L. Ma, Ultrasensitive electrochemical detection of miRNA-21 by using an iridium (III) complex as catalyst, Biosens. Bioelectron. 86 (2016) 454–458.
[34] Q.M. Feng, Y.Z. Shen, M.X. Li, Z.L. Zhang, W. Zhao, J.J. Xu, H.Y. Chen, Dual-wavelength electrochemiluminescence ratiometry based on resonance energy transfer between Au nanoparticles functionalized g-C3N4 nanosheet and Ru (bpy) 32+ for microRNA detection, Anal. Chem. 88 (2015) 937–944.

Please cite this article as: N. Fakhri et al., Paper based colorimetric detection of miRNA-21 using Ag/Pt nanoclusters, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, https://doi.org/10.1016/j.saa.2019.117529