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Research Article

Development of Array-Based Gold Nanoclusters for Discrimination of CA125 Overexpressed Serum Samples

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Here, we reported a simple method for recognizing CA125 overexpressed serum samples using array-based fluorescent gold nanoclusters (AuNCs). The sensing array was fabricated by the combination of various CA125 aptamer functionalized AuNCs. The fluorescence of different aptamer-capped AuNCs was quenched to a different extent in the presence of CA125. By comprehensive analysis of these fluorescence changes, unique patterns were formed when CA125 was overexpressed in serum samples. This strategy is successfully used for discriminating CA125 overexpressed human serum samples, which have great importance for the diagnosis of ovarian cancer in the early stage.

1. Introduction

Ovarian cancer has shown a serious threat to women's health, accounting for ca. 5% of all cancer deaths in females [1]. Early diagnosis of ovarian cancer has great importance for saving lives. For instance, the detection of tumor markers can predict ovarian cancer in the early stages [2]. Among the tumor markers, CA125 has been used by the Food and Drug Administration (FDA) as a clinical tumor marker to predict ovarian cancer. The normal blood level of CA125 in a healthy subject is usually less than 35 U/mL, but CA125 is overexpressed in the blood of most ovarian cancer patients. Various methods have been used for the detection of CA125, such as ELISA (enzyme-linked immunosorbent assay), chemiluminescent sensors, piezoelectric sensors, and electrochemical sensors [3–5]. Most of these detection methods require complicated procedures to prepare the sensors [6]. Biosensors have attracted great attention for the detection of CA125 because they have the potential to be developed as point-of-care devices [7]. For instance, Nunna et al. attached gold nanoparticles on interdigitated electrodes as a biosensor for electrochemical detection CA125 within the concentrations of 3500–84000 U/mL[8]. Hamd-Ghadareh et al. developed blue fluorescent carbon dots (CDs) functionalized with CA125 aptamer, and green fluorescent PAMAM-dendrimers/AuNPs were used at the same time. These two fluorescence sources with different colors were fabricated for generating fluorescence resonance energy transfer (FRET) phenomena between CDs and AuNPs. Such FRET sensor achieved a much more sensitive measurement (0.5 fg/mL) of CA 125 with the range 1.0 pg/mL to 1.0 ng/mL [9]. However, the repeatability of a relatively complicated method may be a concern. The exploration of more repeatable and reliable techniques to detect the abnormal expression of CA125 in the blood is still in requirement.

AuNCs have been used as green fluorescent sensors showing advantages such as low toxicity and easy preparation [10, 11]. Despite their potential for analysis

applications such as metal ions, organic drugs, and environmental pollutants [12–15], they have been rarely used for the detection of tumor markers. This is possible because researchers mainly focus on the development of specific sensors. Normally, a specific sensor relies on a specific bioreceptor to specifically analyze one analyte at one time. In addition, the specific detection of a tumor maker by AuNCs requires complicated functionalization processes [16]. On the other hand, array-based sensors, which are also called "chemical nose" methods [17], have been used for the detection of cancers [18]. A sensor array consists of combined sensing elements, whose interactions with various analytes can generate unique patterns, which can discriminate analytes by linear discriminant analysis (LDA), i.e., a method used in statistics to find a linear combination of features that separates different classes of objects. AuNCs have been used for constructing array-based sensors utilizing charge transfer-induced fluorescence changes. However, AuNC-based arrays have never been used for differentiation of tumor markers either possibly because the charge transfer system cannot show significant responses in such cases [19].

Aptamers can specifically interact with the tumor markers and induce remarkable responses. However, aptamers attached closely to fluorophores are hardly applied for discrimination of certain types of samples because the fluorescence is difficult to be changed [20]. Herein, various aptamers were mixed with AuNCs, and the array-based sensors were constructed directly for differentiating samples with different CA125 levels. Aptamers initially interacted with AuNCs loosely and induced their morphology change due to the soft aggregations. Various AuNC-based systems provide different fluorescent responses such as response 1, response 2, and response 3 (Figure 1). Initially, the aptamer-modified AuNCs could be placed on a sensing pad and show the fluorescence signal changes depending on the interaction force between the aptamer and AuNCs. In the presence of CA125, AuNCs were competed to CA125 to interact with these aptamers. Once CA125 has a stronger interaction force with the aptamers, the fluorescence changes again. These different changes enable the formation of different factors such as factor 1, factor 2, and factor 3 with different patterns. That is to say, AuNCs can finally have different fluorescence intensities after the interaction with the same concentration of CA 125 because the intermediate aptamer is different. Next, LDA can differentiate the CA125 factors in a liquid such as the overexpressed serum samples. To the best of our knowledge, this is the first report for constructing AuNCs as array-based sensors for the detection of tumor markers.

2. Experiments

Transmission electron microscopy (TEM) was performed by using a JEOL JEM-2100 microscope operating at 200 kV. The fluorescence was obtained using a microreader (Varioskan LUX). Serum samples of CA125 with different levels were obtained from the first afflicted hospital of Jinzhou Medical University. CA125 was clinically detected by the chemiluminescent system (ARCHITECT i4000). The aptamers used were designed with the modification according to references [21, 22] which are listed in Table 1 and were obtained from Sangon Biotech (Shanghai) Co., Ltd. All other reagents were obtained from Aladdin company, which were of analytical grades. Deionized water was used through the experiments.

For the synthesis of AuNCs, 100 μL of 150 mM glutathione (GSH) and $HAuCl_4$ (20 mM, 0.50 mL) were mixed with 4.35 mL of deionized water at room temperature. The mixture was transferred to a water bath and heated at 70°C for 24 h. The array-based sensors were obtained by adding 10 μL of (2 mM) AuNCs into the PBS solutions (10 mM, pH 7.4). Various CA125 aptamers (10 μL, 100 nM), as shown in Table 1, were added into different AuNCs solutions, respectively. The mixtures were carried for 1 hour. Then, serum samples of CA125 with different levels were combined with the above solutions, respectively, and the reaction was allowed to process at room temperatures for 1 hour. The fluorescence intensity was measured, and LDA was used to differentiate these samples.

3. Results and Discussion

TEM was used to confirm the formation of AuNCs (Figure 2). It can be seen that the clusters were homogeneously distributed (Figure 2(a)), and the average size (Figure 2(b)) was around 1.4 nm. This indicates that the ultrasmall AuNCs were successfully fabricated.

TEM (Figure 3) was further used to check AuNCs in the absence and presence of the aptamers and CA125. Compared with Figure 2 that revealed the monodispersity of AuNCs in the absence of the aptamers, the aptamer functionalized AuNCs, namely, AuNCs-QS1 (NC1), AuNCs-Q1 (NC2), AuNCs-QN1 (NC3), AuNCs-PS1 (NC4), AuNCs–P1 (NC5), and AuNCs-PN1 (NC6) suffered different aggregations, but most of these changes were insignificant. These aggregations are different from the fused aggregations that result in the formation of large particles. Furthermore, in the presence of CA125, the aptamer functionalized AuNCs all show certain degrees of aggregations or disaggregations, resulting in the quenching or enhancement of the fluorescence to different degrees. By analyzing these comprehensive changes, the system is reliable to reflect the presence of CA125.

For the differentiability analysis, 3 types of artificial bovine serum samples (CA125 > 35 u/mL, Ca125 closed to 35 u/mL, and CA125 < 35 u/mL) were chosen as sensing targets. As illustrated in Figure 4(a), the bovine serum samples with different levels of CA125 resulted in unique fluorescence response patterns due to different interactions with the aptamers, which induce the corresponding morphology change of AuNCs. The fluorescence intensity patterns were subjected to LDA (Figure 4(b)), and different CA125 level samples were well separated. This indicates that the current system can differentiate CA125 overexpressed samples (>35 U/mL) from healthy subjects.

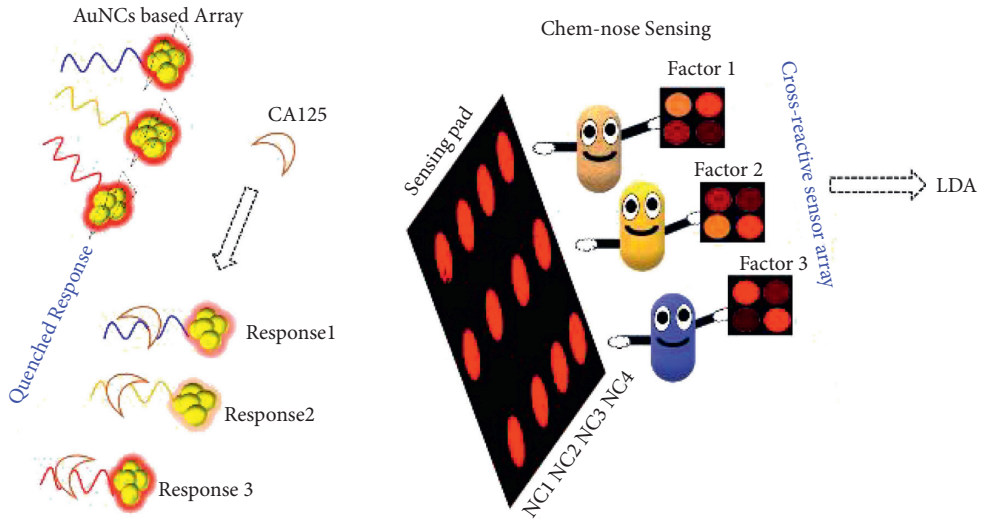

Figure 1: Quenching of the aptamer functionalized AuNCs to different extents and the array-based sensing system for the detection of CA125.

Table 1: Aptamers for constructing the array-based sensing system.

Aptamer	Sequence
QS1	5′-TAATACGA CTCACTATAGGGAGACAAGAATAAACGCTCAA-3′
Q1	5′-SH-(CH2)6TAATACGA CTCACTATAGGGAGACAAGAATAAACGCTCAA-3′
QN1	5′-NH2-(CH2)6TAATACGA CTCACTATAGGGAGACAAGAATAAACG-3′
PS1	5′-ACT TCA GTGAGT TGT CCC ACG GTC GGC GAG TCG GTG GTAG-3′
P1	5′-SH-(CH2)6-ACT TCA GTGAGT TGT CCC ACG GTC GGC GAG TCG GTG GTAG-3′
PN1	5′-NH2-(CH2)6-ACT TCA GTGAGT TGT CCC ACG GTC GGC GAG TCG GTG GTAG-3′

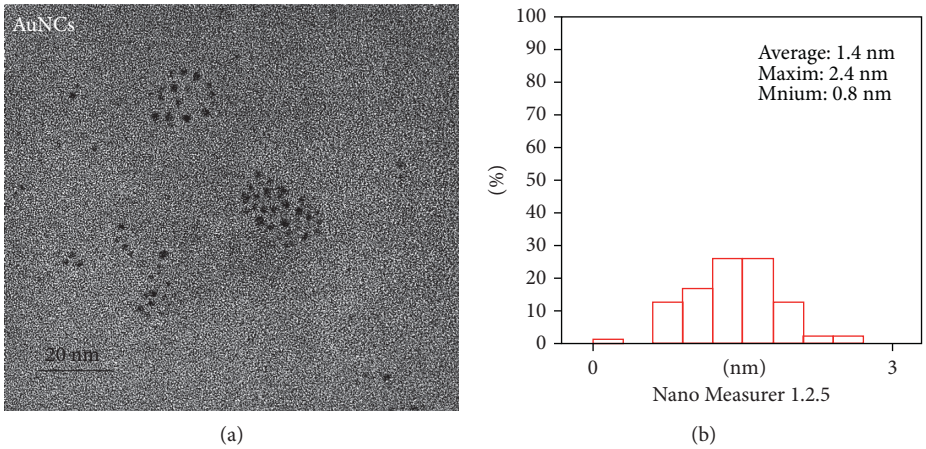

Figure 2: TEM of the original GSH-protected AuNCs.

We tested blinded samples with the AuNC-based sensor arrays. Six unknown human serum samples were randomly selected from the three groups (high, medium, and low levels of CA125) and analyzed by the sensor array (Figure 5) and clinically chemiluminescent methods. As shown in Figure 5(a), different fluorescent responses occurred when the human serum samples of three groups were combined with the sensor array. LDA revealed that serums of CA125

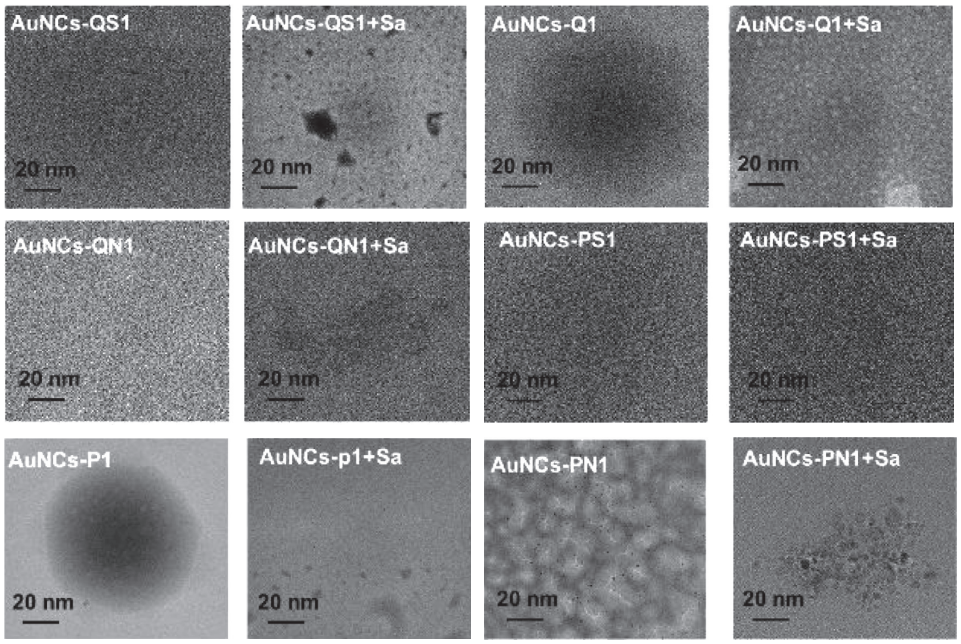

Figure 3: TEM of AuNCs in the presence of the aptamers and CA125.

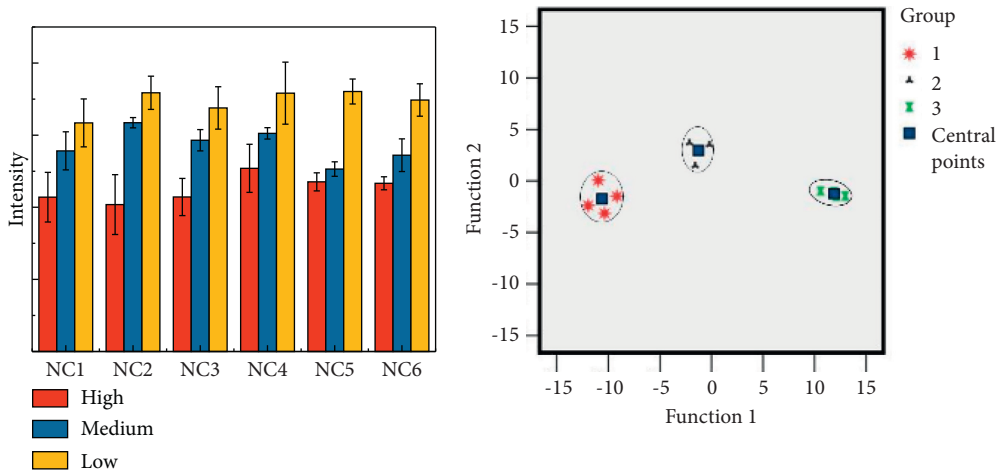

Figure 4: Array-based sensing of artificial bovine serum samples with different CA125 levels. (a) Fluorescence emission intensity (610 nm) patterns of the 3 types of samples (low, medium, and high level of CA125) on the AuNCs array as an average of 4 parallel measurements. (b) LDA shows the excellent separation of the samples with different levels of CA125.

overexpressed samples could be discriminated from those samples with normal CA125 levels (Figure 5(b)). According to the clinically standard results (Table 2), all six serum samples were correctly identified, corresponding to an identification accuracy of 100%. Overall, it could be concluded that the developed sensor array is promising for recognizing CA125 overexpressed serum samples. By comparing with traditional chemiluminometric methods, which were verified by the clinical diagnosis, the results also showed high accuracy and repeatability.

This array sensor can also be used as a specific sensor like the traditional method by selecting one of the aptamer-modified AuNCs. Figure 6 shows the fluorescence spectra (Figure 6(a)) of aptamer 1 (QS1) modified AuNCs, and the corresponding fluorescence change (Figure 6(b)) could be used as the calibration graph for linear detection of CA125 antigen. Similarly, the other aptamer-modified AuNCs all have their calibration graphs. Herein, we combined with aptamer-modified AuNCs for the group-sensing of the analytes. Multiple sensor elements were used for the

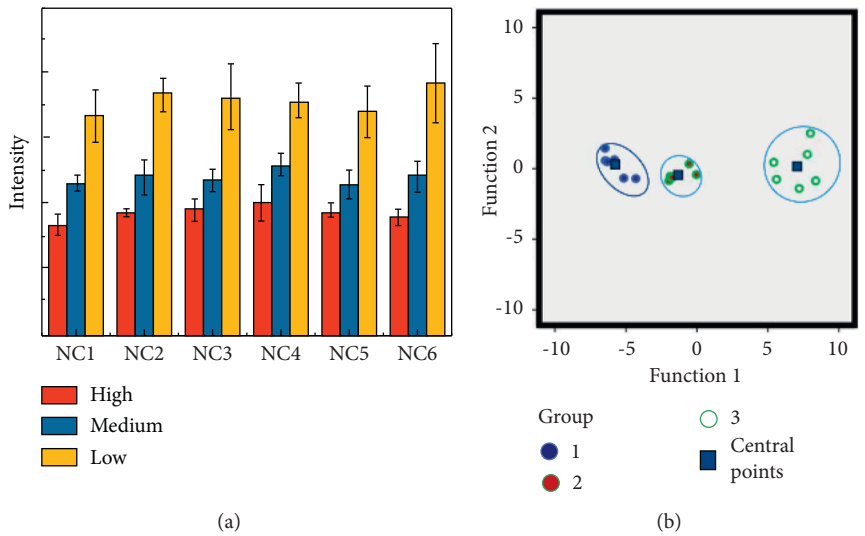

Figure 5: Array-based sensing of human serum samples with different CA125 levels. (a) Fluorescence emission intensity (610 nm) patterns of the 3 types of samples (low, medium, and high level of CA125) on the AuNCs array as an average of six patient samples. (b) LDA shows the excellent separation of the samples with different levels of CA125.

Table 2: Determination of CA125 by the traditional chemiluminescence method.

Test	High	Medium	Low
1	>1000	29.7	<20
2	151.4	28.9	<20
3	294.9	30.8	<20
4	355.4	29.7	<20
5	>1000	24.9	<20
6	882.6	29.1	<20

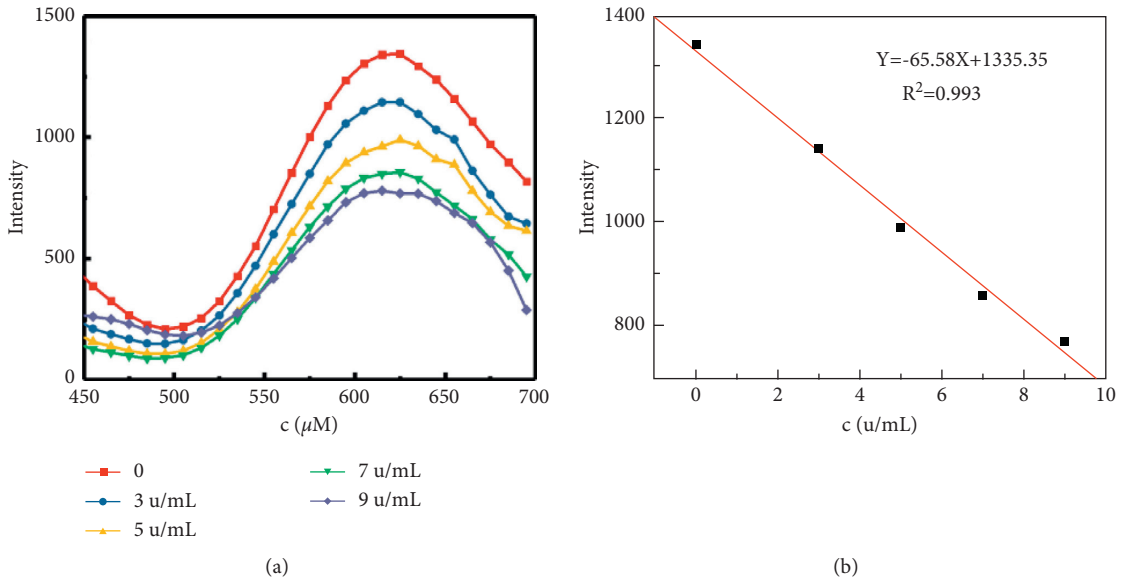

Figure 6: Fluorescence spectra (a) and normalized fluorescence intensity at 610 nm (b) with the increases in concentrations of CA125 from 0 to 9 u/mL; the excitation wavelength was 365 nm

detection of CA125 at the same time, which could provide more accurate data. Because of the repeated verification of multiple factors, this sensor array will have unique reproducibility. Such a design will also provide a robust strategy for various other biomarkers.

4. Conclusions

In this study, we developed a sensor array using six kinds of fluorescent AuNCs with the corresponding aptamers as sensing receptors for CA125 discrimination. Different levels of CA125 expressed samples can be distinguished from each other in human serum samples. This work opens a path for constructing sensor arrays for the diagnosis of tumor markers in clinical practice.

Data Availability

The data generated or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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