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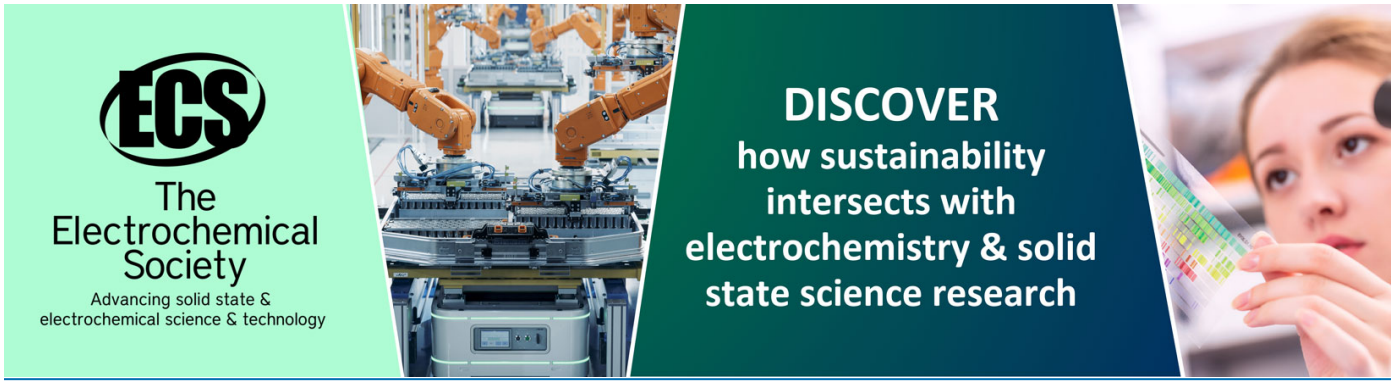

Golgi protein 73 colorimetric biosensor based on reduced graphene oxide-trimanganese tetroxide nanozyme

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Abstract: Golgi protein 73 (GP73) is a new type of marker discovered in recent years for Hepatocellular carcinoma (HCC) detection. Herein, a sandwich colorimetric sensor was constructed for detection GP73 based on the good catalytic activity of reduced graphene oxide-trimanganese tetroxide (RGO-Mn_3O_4) nanozyme labeling GP73 aptamer, GP73 antibody and GP73. RGO-Mn_3O_4 with good peroxidase-like activity could catalyze the oxidation of TMB by H_2O_2 to produce a blue oxidation product (oxTMB) with an absorption peak at 652 nm. In the range of 10-250 ng/mL, different concentrations of GP73 have a linear relationship with absorbance. The catalytic enhancement allows for sensitive colorimetric detection of GP73. The linear regression equation is Y=0.3648+0.0013X with R^2 of 0.9926 and LOD of 5.41 ng/mL. All the experimental results show that the detection system has a lower detection limit and good specificity for the detection of GP73, which provides a new method for the detection of HCC.

1. Introduction

Golgi protein 73 (GP73) is the most promising tumor marker for early diagnosis of HCC discovered in recent years. It has little or no expression in normal liver tissues; in chronic liver diseases such as hepatitis and cirrhosis, it is found in the serum of patients the content of GP73 increased, especially in HCC patients, the expression is particularly significant [1]. Therefore, looking for a simple, convenient and fast method to identify GP73 is of great significance for the early diagnosis of HCC.

The colorimetric sensor is measured by the color change before and after the enzyme oxidation reaction [2]. However, natural enzymes have harsh preparation and storage conditions and are difficult to be widely used. Manganese tetroxide (Mn_3O_4) is a metal oxide with peroxidase-like properties, which can catalyze H_2O_2 and then oxidize the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) to a blue oxidation product (oxTMB) [3-5]. However, Mn_3O_4 has the disadvantage of easy polymerization. reduced graphene oxide (RGO) is a honeycomb-shaped two-dimensional single-layer dense carbon atom, which is widely used due to its large specific surface area, better biocompatibility and easy functionality. Therefore, using RGO as a substrate to combine with Mn_3O_4 to form RGO-Mn_3O_4, which can not only prevent the polymerization of Mn_3O_4 but also further increases the specific surface area and catalytic activity.

2. Materials and methods

2.1 Chemicals and reagents

GP73, GP73 antibody, GP73 aptamer, and alpha-fetoprotein (AFP) were all purchased from Shanghai

Bio-Biotech Co., Ltd. (Shanghai, China); Manganese chloride ($MnCl_2·4H_2O$), 3,3',5,5'-tetramethylbenzidine (TMB), o-phenylenediamine (OPD) was purchased from Macleans Biochemical Technology Co., Ltd. (Shanghai, China). All the solutions were prepared with ultrapure water of 18 Ω·cm purified from a Milli-Q purification system (Milli-Pore, Bedford, MA, USA). The human blood serum was acquired from 181st Hospital of Chinese People's Liberation Army (Guilin, China).

All experiments were performed on ultraviolet visible spectroscopy were made in the wavelength range of 500-800 nm (UV-vis, UH5300, HITACHI, Japan).

2.2 Preparation of RGO-Mn_3O_4-Apt signal probe

Reductive graphene oxide (RGO) was prepared according to the Huang et al [6] method; First, 445 mg of $MnCl_2·4H_2O$ was added to 10 ml of ultrapure water and stirred to dissolve; 500 mg of polyvinylpyrrolidone (PVP) was added to the solution, and a glass rod was used to stir and mix. add 40 ml of ultrapure water (85 ºC) and add 180 mg of sodium hydroxide (NaOH), stir in a water bath at 85 ºC for 3 h to obtain a Mn_3O_4 solution. Second, weigh 1 mg of NHS and 4 mg of EDC into 1 ml of RGO solution, meanwhile, add 300 μl of Mn_3O_4 solution with stirring at room temperature, and stir at room temperature for 12 h with a stirrer to obtain RGO-Mn_3O_4 nanozyme. Finally, the 1.0 μg/ml RGO-Mn_3O_4 nanoenzyme material and the 10.0 μg/ml GP73 aptamer solution were mixed at a ratio of 1:2 and incubated overnight to obtain the RGO-Mn_3O_4-Apt signal probe.

2.3 Detection of GP73 based on RGO-Mn_3O_4 nanoenzyme colorimetric sensor

First, add 20 μl, 10 μg/ml of antibody the test tube; second, add 20 μl of BSA blocking solution and incubate for 2 h at room temperature to prevent non-specific binding; third, add 20 μl of antibody at a concentration of 10 μg/ml. 5 μg/ml GP73 was incubated at room temperature for 2 h; finally, 20 μl of RGO-Mn_3O_4-Apt detection probe was added and incubated at room temperature for 1 h. Then 10 μl of TMB and H_2O_2 solutions were added separately, and the resulting solution was measured with a UH5300 ultraviolet-visible spectrophotometer. Record the absorption peak at 652 nm.

3. Results and discussion

3.1 Preparation of RGO-Mn_3O_4 nanoenzyme and RGO-Mn_3O_4-Apt signal probe

Figure 1A is the XRD diagram of RGO-Mn_3O_4 nanoenzyme, curve a shows the XRD characterization of Mn_3O_4. There are sharp diffraction peaks at 2θ=18°, 36.08°, and 60.63°, which are different from each other. (101), (211), (215) correspond, the positions of other diffraction peaks correspond to the standard card (JCPDS 24-0734); curve b is the XRD characterization of RGO-Mn_3O_4 nanozyme, RGO has a weak broad diffraction peak at 2θ=18~22°, which belongs to the diffraction surface of graphene material C (002). Due to the addition of Mn_3O_4, the aggregation and re-stacking of the graphene composite material is prevented, and the regular interlayer spacing of the graphene sheet is reduced, so there is no obvious characteristic peak [7]. The rest of the diffraction peaks correspond to the standard card one-to-one. The above phenomena all indicate that the RGO-Mn_3O_4 nanozyme was successfully prepared.

The UV-vis spectroscopy of RGO, Mn_3O_4, RGO-Mn_3O_4, NH_2-GP73 and singal probe are shown in Figure 1B. The maximum absorption of rGO at 265 nm (Figure 1B, curve a) is due to the π-π* transition of the aromatic C=C bond. Mn_3O_4 (Figure 1B, curve b) has an absorption maximum at 205 nm, while RGO-Mn_3O_4 (Figure 1B, curve c) has absorption peaks at 260 nm and 200 nm, respectively. The red shift to the left indicates the sp^2 structure of rGO recovery. This finding indicates that the RGO-Mn_3O_4 nanozyme has been successfully prepared. In addition, NH_2-$GP73_{Apt}$ (Figure 1B, curve d) has a maximum absorption peak at 260 nm and the signal probe (Figure 1B, curve e) have absorption peaks at 260 nm and 205 nm. The calculated binding rate of the signal probe is 86%, indicating that the RGO-Mn_3O_4-Apt signal probe has been successfully prepared.

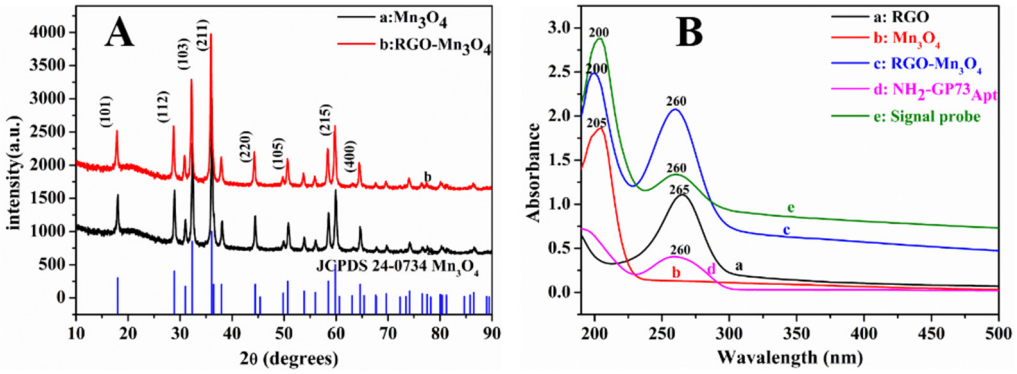

Fig.1 (A) XRD characterization of Mn_3O_4 (curve a), RGO-Mn_3O_4 (curve b); (B) UV-vis characterization of RGO (curve a), Mn_3O_4 (curve b), RGO- Mn_3O_4 (curve c), NH_2-GP73(curve d), Signal probe (curve e)

3.2 Principle of colorimetric GP73 biosensor based on RGO-Mn_3O_4 nanoenzyme

The principle diagram of the colorimetric GP73 biosensor based on RGO-Mn_3O_4 nanozyme is shown in Figure 2. The RGO-Mn_3O_4-Apt signal probe was synthesized by a step-by-step method. The GP73 antibody was used as the capture probe. When GP73 was present, the GP73 antibody and GP73 aptamer captures GP73 at the same time to form a sandwich structure. RGO-Mn_3O_4 nanoenzyme falls off, catalyzes the conversion of H_2O_2 to H_2O and O_2, while TMB converts to oxTMB in the presence of O_2. The solution changes from colorless to blue, and an UV-vis was used to observe the change of the absorption peak at 652nm, so as to realize the detection of GP73.

Figures 2B and Figures 2C respectively show the feasibility of experimental phenomena and absorbance maps. From Figure 2B, it can be seen that tube 1 is H_2O_2 and does not decompose in air. Therefore, there is no absorbance, and tubes 1-4 are light blue, This is because TMB will be partially oxidized in the presence of oxygen in the air. Therefore, tubes 1-4 have lower absorbance, while tube 5 has the darkest color; The color of tube 5 is the darkest; its corresponding absorbance spectrum curve (Figure 2B), curve 5 has the highest absorbance, that is, when Ab, GP73 and Apt coexist in the system, the absorbance of the system is the strongest, indicating that the GP73 colorimetric sensor based on RGO-Mn_3O_4 nanozyme can be used for the detection of GP73.

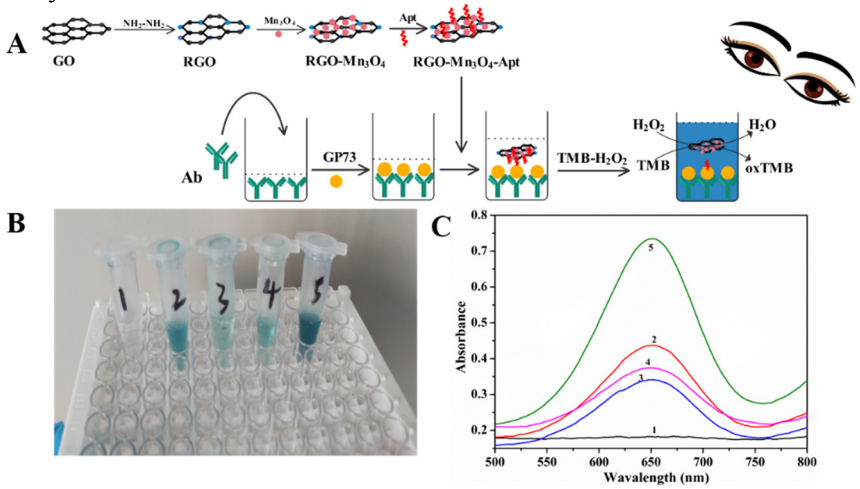

Figure 2. (A) Schematic diagram of colorimetric GP73 biosensor based on RGO-Mn_3O_4 nanozyme; (B) Feasibility experiment picture of GP73 colorimetric biosensor based on RGO-Mn_3O_4 nanozyme; (C) Absorbance of feasibility of TMB colorimetric cholesterol biosensor based on HGNs(1 is TMB+H_2O_2, 2 is Ab+RGO-Mn_3O_4-Apt+TMB-H_2O_2, 3 is GP73+RGO-Mn_3O_4-Apt+TMB-H_2O_2, 4 is RGO-Mn_3O_4-Apt+TMB-H_2O_2, 5 is Ab +GP73+RGO-Mn_3O_4-Apt+TMB-H_2O_2)

3.3 Performance analysis of GP73 colorimetric biosensor based on RGO-Mn_3O_4 nanozyme

Under the optimal conditions, the GP73 colorimetric biosensor based on RGO-Mn_3O_4 nanozyme was used to detect GP73. The results are shown in Figure 3. Figure 3A shows the color change of the solution at different GP73 concentrations. It can be seen that the higher the GP73 concentration, the darker the color; and from Figure 3B, it can be seen that as the concentration of GP73 increases, the absorbance increases, the absorbance and the concentration of GP73 showed a linear relationship between 10~250 ng/mL. The result is shown in Figure C. The linear regression equation is Y=0.3648+0.0013X, and the correlation coefficient R^2=0.9926. And according to the equation C_{LOD}=3*Sb/b (Sb is the standard deviation, b is the slope of the standard curve), the detection limit is calculated to be 5.419 ng/mL.

In addition, BSA, AFP, HAS, and IgG were used to perform interference tests. The results showed that the four targets of BSA, AFP, HAS, and IgG had less interference to GP73, indicating that the RGO-Mn_3O_4 nanozyme-based GP73 colorimetric biosensor has better performance. With high specificity and selectivity, it can be used for the detection of GP73.

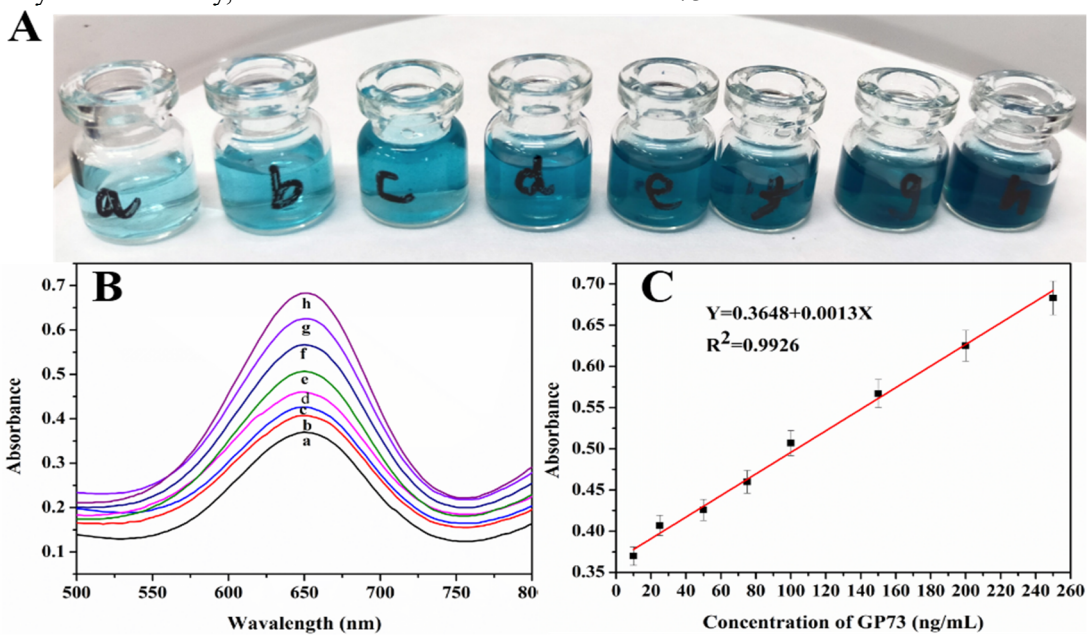

Figure 3. (A) Experimental phenomenon of different concentrations of GP73; (B) Absorbance curve of different concentrations of GP73;(C) Standard curve of GP73 at different concentrations

3.4 Detection of GP73 in human serum samples

A restoration test was conducted by spiking GP73 at diverse concentrations into the truthful human serum sample under optimal experimental prerequisite to verify the applicability of this colorimetric sensor in authentic substrates. The consistency of GP73 after three diverse concentrations (150, 200 and 250 μg/mL) of GP73 standard solution subjoined serum samples was detected using UV-vis, and the results are displayed in Table 1. The recovery rate of the colorimetric sensor is between 96.65%-122.57%. The RSD value is between 0.18-3.05% (n=3). These measurement data are satisfactory, verifying the accuracy and reliability of the sensor in real samples.

Table 1 Actual serum test results (Measured (n=3))

	GP73 Added (ng/mL)	Average of Detection concentration (ng/mL)	RSD (%)	Recovery (%)
Sample 1	150	155.10	1.48	103.40
	200	201.42	0.95	100.71
	250	241.62	0.18	96.65
Sample 2	150	162.99	3.05	108.66
	200	213.63	1.61	106.82
	250	257.65	0.62	103.06
Sample 3	150	183.86	1.20	122.57
	200	220.50	1.31	110.25
	250	268.08	0.75	107.23

4. Conclusions

In summary, a highly sensitive GP73 colorimetric biosensor was constructed based on the good peroxidase-like activity of RGO-Mn_3O_4 nanozyme. In the range of 10-250 ng/mL, the absorbance is linearly related to the GP73 concentration, the regression equation was Y=0.3648+0.0013X with R^2 of 0.9926. The colorimetric biosensor also has a great recovery rate (96.65%-122.57%) in the detection of human serum samples. This indicates that GP73 colorimetric biosensor has great potential in clinical diagnosis.

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