A highly specific and sensitive electroanalytical strategy for microRNAs based on amplified silver deposition by the synergic TiO$_2$ photocatalysis and guanine photoreduction using charge-neutral probes

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TiO$_2$ photocatalysis and guanine photoreduction were synergically combined for amplifying silver deposition for the electroanalysis of short-chain microRNAs with guanine bases using charge-neutral probes. It could allow for the highly specific and sensitive detection of microRNAs in the blood as well as the identification of their mutant levels.

MicroRNAs (miRNAs) are non-coding short-chain RNAs with approximately 18–25 nucleotides in length. They are useful diagnostic and prognostic markers for many basic biomedical research studies and evaluations. In particular, the expression levels of miRNAs in peripheral blood have been well established to be sensitive biomarkers for cancer diagnostics and metastasis. The quantification of free miRNAs in the blood, however, can be challenged by many factors like the short chain length and low expression levels of miRNAs. For example, the common sandwiched detection of short-chain miRNAs of low melting temperature might in a way risk the unwinding of hybridized sequences. Therefore, exploring highly sensitive analysis strategies and suitable efficient detection modes for the analysis of short-chain miRNA targets is of great interest.

In recent decades, many modern analytical methods have been established to quantify or identify miRNA levels, the most known methods being the northern blotting, electrochemical biosensing, reverse transcription polymerase chain reaction, and surface enhanced Raman spectroscopy. Among these classical detection methods, electrochemical biosensing has been recognized as a particularly attractive alternative with high analysis sensitivity, low detection limit, fast response time, cost effectiveness, and ease of automation. For example, Dong and co-workers described a label-free electrochemical biosensor for the detection of miRNAs with the limit of detection down to 67 fM. Gao’s group has reported the electrochemical analysis of miRNAs using some special catalyst labels of metal oxides. A sandwiched electroanalytical protocol has also been developed for the electroanalysis of low-level miRNAs in blood based on silver deposition catalyzed by a gold nanocluster-incorporated enzyme. Noticeably, the catalytic silver deposition procedure has been widely applied as a powerful signal amplification tool, nevertheless, a common challenge can be mostly encountered regarding the non-specific adsorption of positively charged silver ions onto the oppositely charged phosphodiester backbone probes of nucleic acids. Alternatively, many efforts have been devoted to the use of some nonionic nucleic acid analogs, such as peptide nucleic acids (PNAs) and morpholinos, in the solid-phase assays for nucleic acids. Especially, PNAs with charge-neutral pseudo-peptide backbones could serve as outstanding probe candidates for the specific molecular recognition of nucleic acid targets in biosensor design.

Moreover, titanium dioxide (TiO$_2$), a photocatalyst with excellent chemical stability, low toxicity, low cost, and redox properties, has been extensively applied for solar cells and photocatalytic cleanup of toxic organics in wastewater. In 1997, Dunford and colleagues demonstrated that TiO$_2$ particles could catalyze the DNA damage in vitro or in cells. Also, Wamer et al. found that guanine, an easily oxidized DNA base, could play a vital role in the photo-oxidative damage of DNA oligonucleotides physisorbed on TiO$_2$ particles. More importantly, Rajh and co-workers discovered an efficient light-induced crosstalk across the TiO$_2$ interface and DNA oligonucleotides with guanine bases for the photocatalytic deposition of metallic silver.

Inspired by the pioneering work mentioned above, in the present study, a synergic combination of TiO$_2$ photocatalysis and guanine photoreduction has been proposed for the first time to amplify the silver deposition towards a highly sensitive and selective analysis method tailored for probing guanine-containing miRNAs of hsa-let-7a in blood using charge-neutral PNA probes. Here, the miRNA sequences from the hsa-let-7 family,
of which the aberrant expression levels can be associated with the diagnosis and prognosis of cancers. The main detection principle and procedure are schematically illustrated in Scheme 1. As shown in Scheme 1A, magnetic hollow Fe₃O₄ particles were first synthesized using a modified solvothermal method, and then coated with TiO₂ shells by using a modified sol–gel method, resulting in Fe₃O₄@TiO₂ nanocomposites with magnetic separation and photocatalysis functions. Scanning electron microscopy (SEM) imaging was conducted to characterize the resulting Fe₃O₄ particles before and after TiO₂ coating (Fig. 1A). It was found that Fe₃O₄ particles could display a uniformly defined spherical shape with an average size of about 500 nm. Furthermore, as shown in Fig. 1B, TiO₂ layers could be coated onto Fe₃O₄ particles with compact and lumpy structures, and the formed Fe₃O₄@TiO₂ nanocomposites showed an average size of about 570 nm. The crystalline structures of Fe₃O₄ spheres and Fe₃O₄@TiO₂ nanocomposites were further investigated using powder X-ray diffraction (XRD). One can note from Fig. 1C that the marked diffraction peaks of crystalline Fe₃O₄ can be obtained in good agreement with the well established data (JCPDS 75-1609). Importantly, the typical XRD pattern of crystallographic planes marked for anatase-phase TiO₂ could be additionally attained for Fe₃O₄@TiO₂ nanocomposites (Fig. 1D).

The analytical performances of silver deposition-based electroanalysis of miRNAs containing guanine bases were comparably investigated, for which the different detection conditions are schematically illustrated (Fig. 2). As shown in Fig. 2A, the electrochemical silver response is much larger in the visible light (Curve a) than in the dark (Curve b), thus confirming that stronger photocatalytic silver deposition could be achieved under the visible light that would be chosen as the necessary condition for the detection of miRNA targets. Furthermore, the silver deposition signal could be neglected for the PNA probes alone, indicating that PNA probes as nonionic nucleic acid analogs could present non-significant adsorption of Ag⁺ ions (Curve c). Importantly, it demonstrates that the photocatalytic silver deposition might not occur in the absence of the targeting miRNAs with the photoreducing guanine bases. Moreover, Fig. 2B discloses that Fe₃O₄@TiO₂-DA (Curve a) could exhibit a much greater silver response than Fe₃O₄-DA without TiO₂ (Curve b), indicating that the TiO₂ photocatalyst could play a vital role in the amplified silver deposition.

The Fe₃O₄@TiO₂-DA dosages were optimized for the electrochemical silver responses, showing that 1.0 mg mL⁻¹ of
Fe₃O₄@TiO₂-DA is enough (Fig. S1A, ESI†). Interestingly, too high concentrations of Fe₃O₄@TiO₂-DA might lead to slightly decreased signals, presumably due to the fact that the non-conductive nanocomposites could be overlaid on the electrode surfaces with too high density so as to conduct the negative effects on the electrochemical silver responses. Also, the influence of the amounts of Ag⁺ on the photocatalytic silver deposition was experimentally investigated, showing that the peak currents of silver responses could reach the constant at 1.0 mM AgNO₃ (Fig. S1B, ESI†). Furthermore, the time for the probe-target hybridization and photocatalytic silver deposition reactions was separately explored. As shown in Fig. S1C (ESI†), the electrochemical silver responses could increasingly tend to be steady after 50 min, which was thus chosen as the hybridization reaction time. In addition, Fig. S1D (ESI†) displays that the photocatalytic silver deposition reactions occur very fast, with the saturated silver signals obtained at about 5.0 min.

Under the optimized experimental conditions, the silver deposition-based electroanalytical method was employed to detect the wild and mutated miRNA samples (Fig. 3). Fig. 3A shows the electrochemical responses to wild miRNA targets of different concentrations in buffer. A linear current correlation to the logarithm of the miRNA concentrations was obtained across the concentration range from 2.0 fM to 2.0 nM (R² = 0.9947), with the detection limit of 1.3 fM, estimated according to the 3σ rule (Fig. 3B). In the meantime, the developed electroanalytical method was applied to probe the levels of wild miRNAs spiked in blood samples with different concentrations (Fig. 3C). A linear detection relationship between the electrochemical responses and wild miRNA levels in blood was obtained over the miRNA concentrations ranging from 2.0 fM to 2.0 nM (R² = 0.9833), with the detection limit of about 1.6 fM. Moreover, the electrochemical responses of the silver deposition to the single-base and double-base mismatched miRNAs were examined by comparing with the wild miRNA targets (Fig. 3D). As expected, the single-base mismatched miRNAs (Curve b) showed much lower responses than the matched ones (Curve a), whereas the double-base mismatched targets could manifest the lowest responses (Curve c), as also illustrated in the inset of Fig. 3D. Accordingly, the as-developed electroanalytical method enables the discrimination of mutation levels of the single-base and double-base mismatched miRNAs. Herein, the mutations of single and double guanine bases of miRNAs might decrease the guanine-enabled photoreduction abilities for silver deposition at different degrees. That is, the photoreduction of guanine bases of miRNAs is highly needed for the photocatalytic silver deposition aforementioned. In addition, the existence of mismatches in the miRNA might additionally make an insulating barrier to prevent the hole migration to the guanine–guanine accepting sites, leading to a decrease in the TiO₂ photocatalysis for the silver deposition, as also demonstrated elsewhere.²⁵,²⁶ Therefore, the developed silver deposition-based electroanalysis strategy could allow for the detection of wild miRNA targets in blood with high detection sensitivity and selectivity. Also, it can allow for the quantification of mutant levels of miRNA targets for profiling the RNA expression pattern.

To summarize, in this work, a highly specific and sensitive electroanalytical methodology has been successfully developed for probing guanine-containing miRNA targets in blood based on the amplified silver deposition by the synergic combination of TiO₂ photocatalysis and guanine photoreduction using charge-neutral probes. This silver deposition-based electroanalytical method possesses some outstanding advantages over most of the traditional detection methods for short-chain miRNAs. First, the synergic combination of the photocatalysis of TiO₂ and the photoreduction of guanines of targeting miRNAs could achieve the dramatically amplified signal for silver deposition, which promises a highly sensitive detection of miRNAs specifically containing guanine bases. Second, the coatings of DA on Fe₃O₄@TiO₂ nanocomposites could endow the shelled TiO₂ nanoparticles...
with tunable photocatalytic responses in the visible light of great interest, in addition to the covalent loading of captured probes. Third, the use of charge-neutral PNAs might overcome any non-specific adsorption of silver ions to facilitate the highly selective detection of miRNAs. Fourth, the introduction of magnetic carriers could allow for the convenient separation of miRNAs from the complicated blood samples without the tedious purification steps. Finally, the sensitive electrochemical output of silver deposition with magnetic electrodes could facilitate the sensitive detection of miRNAs. Therefore, the developed electroanalytical method could not only probe guanine-containing miRNAs in blood with high sensitivity and selectivity, but also identify their single-base and double-base mutation levels. Importantly, such an electroanalytical mode may be tailored for the short-chain nucleic acids containing reducing guanines. It holds great promise for wide applications in the clinical laboratory for cancer diagnosis and early warning of cancer metastasis.

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Notes and references