Low Background Electrochemical Sensor based on HCR Towards Acute Myocardial Infarction-specific miRNA Detection

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Optimization of the fractal gold electrode

To investigate the impact of deposition potential on the morphology and electrochemical performance of the gold electrodes, various morphological structures were created on screen-printed electrodes (SPEs) using electrodeposition techniques. Electrodepositions were conducted for 500s using 100 μ L of 10 mg/mL HAuCl₄ solution under different deposition potentials. The gold electrodes exhibited diverse sizes, shapes, and electrochemical properties. As shown in Fig. S1A, at a potential of - 0.3 V, spherical gold nanoparticles were evenly distributed on the electrode surface. As the voltage became to -0.5 V, the morphology of the gold particles changed, transitioning from spherical to irregular shapes, accompanied by particle aggregation. At -0.7 V, branched structures started emerging on the electrode surface, albeit less densely. At -1.0 V, the branched gold nanostructures exhibited increased complexity, with initial signs of interconnected network formation. At -1.2 V, a well-defined fractal branched gold structure with symmetry appeared. However, compared to the branched gold morphology under -1.5 V, the structure at -1.2 V was less refined and dense.

Additionally, the electrochemical characterization of cyclic voltammetry (CV) and the differential pulse voltammetry (DPV) were investigated under different deposition voltages. Fig. S1B and C presented the CV and DPV curves at various deposition potentials. Both CV and DPV peak currents increased gradually with increasing voltage, and the maximum electrochemical responses were observed at -1.5 V. It was

crucial to note that excessively high absolute voltage values might lead to potential damage to the SPE. Therefore, considering both the electrode morphology and electrochemical performance, a deposition voltage of -1.5 V was selected as the optimal condition.



Fig. S1 The morphology and electrochemical performance of the gold electrodes under different deposition voltages in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. (A) SEM images of the gold electrode surfaces. CV (B) and DPV (C) curves under different deposition voltage.

Subsequently, an investigation was performed to assess the impact of varying concentrations of HAuCl₄ on the morphology and electrochemical performance of gold electrodes. SEM images of gold nanostructures formed on SPEs after electrodeposition for 500 s at -1.5 V in different concentrations of HAuCl₄ solution (100 μ L) were shown in Fig. S2A. Under low concentration conditions (1 mg/mL), the electrochemically generated fractal gold nanostructures on the electrode surface appeared relatively sparse and uneven. As the concentration of HAuCl₄ increased to 3 mg/mL, the density of the fractal gold structures on the electrode surface gradually increased. At a concentration

of 5 mg/mL, the surface fractal structures appeared denser compared to 1 mg/mL and 3 mg/mL. Continuing to increase the concentration from 7 mg/mL to 10 mg/mL resulted in a gradual stabilization of the fractal gold structure, with large areas of finely structured fractal gold distributed on the electrode surface. Fig. S2B and C showed the CV and DPV curves under different concentrations of HAuCl₄. Both CV and DPV peak currents gradually increased with the increasing concentration of HAuCl₄. When the concentration of HAuCl₄ reached 7 mg/mL, the current intensity reached its maximum value, with no significant variations observed in the CV and DPV signals upon further increasing the concentration. Therefore, considering both the electrode morphology and electrochemical performance, a final concentration of 7 mg/mL of HAuCl₄ was chosen as the optimal deposition electrolyte concentration.



Fig. S2 The morphology and electrochemical performance of the gold electrodes in different HAuCl₄ concentration performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ (5 mM) containing 0.1 M KCl. (A) SEM morphology of the gold electrode surfaces. CV (B) and DPV (C) curves of the gold electrode under different concentrations of HAuCl₄.

The morphology and electrochemical performance of the gold electrode were found

to be significantly influenced by deposition times. As illustrated in Fig. S3A, rough structures were observed on the surface of the bare carbon electrode. After 100 s of deposition, fractal gold structures began to emerge, appearing relatively sparse and lacking well-defined branching structures. With an increase in deposition time, the fractal gold structures gradually became denser, exhibiting more pronounced fractal characteristics. Notably, at a deposition time of 400 s, finely structured fractal gold almost entirely covered the electrode interface. Furthermore, the electrochemical signals (CV and DPV) were investigated at different deposition times. Fig. S3B and C depicted the CV and DPV curves of the electrode, respectively. It was observed that the CV and DPV peak currents on the electrode surface increased gradually as the deposition time increased. Nevertheless, at 400 s, the growth of CV and DPV peak currents slowed down. This decrease in concentration resulted in the chloroauric acid solution becoming almost transparent, indicating the completion of the deposition process. Considering both the electrode morphology and electrochemical performance, an optimal deposition time of 400 s was selected.



Fig. S3 The morphology and electrochemical performance of the gold electrodes under

different deposition times in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. (A) SEM images of the electrode surfaces. CV (B) and DPV (C) curves of the gold electrode under different deposition times.

Surface characterization of the FracAu/SPE

The optimal deposition parameters, including a deposition voltage of -1.5 V, an electrolyte concentration of 7 mg/mL, and a deposition time of 400 s, were selected based on the optimization results of the electrochemical deposition experimental parameters. The rough structures of the bare carbon electrode surface before electrodeposition were illustrated in Fig. S4A. SEM images of the electrode surface after electrochemical deposition were depicted at different magnifications in Fig. S4B-D. The large fractal-like gold nanostructures on the electrode surface, exhibiting high roughness, uniformity, and a three-dimensional structure, were observed in the low-magnification SEM image. At high magnification, the fractal branched gold structures by the aggregation of multiple gold nanoparticles, each approximately 100 nm in size and similar in shape and size, were evident, emphasizing the fine structure of the fractal branched gold.



Fig. S4 SEM images of the electrode surface before and after electrochemical deposition. (A) SEM image of the bare carbon electrode surface before

electrodeposition. (B) Large scale SEM image of the electrode surface after electrochemical deposition at low magnification. (C) High-resolution SEM image of the electrode surface after electrochemical deposition at high magnification. (D) Highresolution SEM image of the electrode surface after electrochemical deposition at high magnification.

Feasibility of the electrochemical sensor based on HCR

To assess the viability of the electrochemical sensor based on HCR for miRNA detection, the theoretical structures and thermodynamic characteristics of the probes (hpDNA, H1, H2) were simulated and evaluated using NUPACK software. The outcomes illustrated in Fig. S5 revealed the respective binding free energies of the three probes to be -18.64 kcal/mol, -19.01 kcal/mol, and -18.72 kcal/mol. These values signified the stability of the designed probe structures and confirmed their presence in a hairpin conformation, without the occurrence of intricate secondary structures.



Fig. S5 Ideal structures and free energy parameters of hpDNA, H1 and H2.

Analytical performance of the sensor without Exo I

The experiments were evaluated both with and without Exo I to evaluate the influence of this enzyme on the performance of the sensor. As illustrated in Fig. S6A, in the absence of Exo I, the current of RuHex significantly increased with rising concentrations of miRNA-483. Within the miRNA-483 concentration range of 10 fM to 10 nM, Fig. S6B demonstrated a substantial linear correlation between I_{Ru} and the logarithm of miRNA concentration, described by the equation I= 0.000899logC + 0.01925 (R² = 0.98941). The detection limit of the sensor was 0.17 pM. Compared to the experiments with Exo I, the linear relationship was less satisfactory and exhibited a higher detection limit in the absence of Exo I. This finding demonstrated that the addition of Exo I could effectively reduce background noise and enhance detection sensitivity.



Fig. S6 Performance of the miRNA electrochemical detection sensor without Exo I. (A) DPV responses at different concentrations of target (10 fM-10 nM) without the presence of Exo I. (B) Linear relationship between DPV signal and logarithm of miRNA-483 concentration without the presence of Exo I. DPVs were performed in PBS solution (10 mM, pH=7.4).





Fig. S7 The full uncropped Gels and Blots images