

## Article

# Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> Nanobelts Electrode for Highly Selective and Sensitive Detection of Cancer miRNAs

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**Abstract:** The design and engineering of effective electrode materials is critical in the development of electrochemical sensors. In the present study, Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts were synthesized by an alkaline hydrothermal process. The structure and morphology were investigated by X-ray diffraction (XRD) and field emission scanning electron microscope (FESEM) measurements. The Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts showed a distinctly enhanced (004) reflection peak and rough surfaces and were used for the electrochemical selective sensing of various cancer miRNAs, such as prostate cancer miR-141, osteosarcoma miR-21, and pancreatic cancer miR-1290. Voltammetric measurements showed an oxidation peak at +0.4, +0.2, and +1.53 V for the three different cancer biomarkers, respectively, with the detection limit as low as 4.26 aM. The results suggest that the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts can be used as active materials to detect early cancers, for in vitro screening of anticancer drugs, and molecular biology research.

**Keywords:** Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub>; miRNA; detection; selective; sensitive; electrochemical

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## 1. Introduction

Cancer is the most threatening disease in human life and health, and cancer research has been attracting extensive attention. As miRNAs are closely related to disease and it is generally believed that changes in nucleic acid molecules are the most critical primordial links of cell carcinogenesis, the research on miRNAs is important for the early diagnosis and immunotherapy of cancer [1]. For instance, recent studies have shown that miR-141 is a potentially useful biomarker for prostate cancer for the up-regulation in prostate cancer specimens [2]. MiR-21 plays an important role in developing osteosarcoma and can be used as a potential serum marker for it [3]. The content of miR-1290 will increase during the development of pancreatic cancer, so the determination of miR-1290 can be exploited for the early detection of pancreatic cancer [4]. However, current miRNA research and detection generally require complex pretreatment steps, expensive optical instruments, and fluorescent reagents [1]. Therefore, it is of great significance to develop rapid, sensitive, simple, and accurate miRNA detection methods that can be used for practical applications in life science research and medical and clinical diagnosis [5].

Nanomaterials have broad applications in the life sciences due to their unique physical and chemical properties and structural characteristics. Of these, titanium dioxide (TiO<sub>2</sub>) nanostructures have been exploited for the sensitive, accurate, rapid, and simple detection and diagnosis of cancer, e.g., nano-p-n junction heterostructures, NiO/TiO<sub>2</sub> nanobelts for the detection of anticancer drugs and biointeractions with cancer cells [6], Au/TiO<sub>2</sub> bi-heterostructured nanobelts for the specific sensing of diverse tumour cells [7], mesoporous TiO<sub>2</sub> for the sensing of pancreatic cancer miR-1290 [8], rutile TiO<sub>2</sub> facet heterojunction nanostructure nuclease for the detection of miR-155 [9], and TiO<sub>2</sub> nanotube arrays for the detection of prostate protein antigen [10], among others.

For electrochemical biosensors, the active sensing material on the electrode acts as a catalyst that promotes the reaction of biochemical molecules to produce output signals [11]. The catalytic activity is the key factor in the selection and development of sensing materials which can be further manipulated by the doping of select rare earth elements [12] or the introduction of oxygen vacancies [13]. Holmium trioxide,  $\text{Ho}_2\text{O}_3$ , is an important rare earth metal (lanthanide) oxide with attractive electrical and optical properties. It has been widely used in environmental protection [14–16], functional ceramics [17,18], and sensing devices [19]. Mortazavi-Derazkola and coworkers [16] synthesized nano- $\text{Ho}_2\text{O}_3$  and observed an outstanding photocatalytic performance. Pan reported a high-k  $\text{Ho}_2\text{O}_3$  sensing film for a pH ion-sensitive field-effect transistor (ISFET) [19]. Additionally, when  $\text{Ho}_2\text{O}_3$  is doped into  $\text{TiO}_2$ ,  $\text{Ti}^{4+}$  is partly replaced by  $\text{Ho}^{3+}$ , leading to the formation of abundant oxygen vacancies in  $\text{TiO}_2$  and hence enhancing bulk conductivity and surface electrocatalytic activity. This will ultimately improve the electrochemical sensing activity of  $\text{TiO}_2$ . However, to the best of our knowledge, there have been no reports on using  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  as active materials to detect cancer miRNAs.

In this work,  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  nanobelts are prepared by using an alkaline hydrothermal process and exhibited high specificity and sensitivity for the electrochemical detection of cancer miRNAs. Specifically,  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  nanobelts can selectively detect miR-141, miR-21, and miR-1290 at distinctly different potentials and achieve a limit of detection as low as 4.26 aM, which suggests that  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  nanobelts may be used as a promising electrode material for early cancer diagnosis, drug screening, and molecular biology research.

## 2. Materials and Methods

### 2.1. Materials

Titania P-25 ( $\text{TiO}_2$ , ca. 75 wt.% anatase and 25 wt.% rutile, Degussa Co., Frankfurt, Germany),  $\text{Ho}_2\text{O}_3$ , and sodium hydroxide, were purchased from China National Reagents Corporation Ltd. (Shanghai, China). MicroRNA oligonucleotides (cDNA) were purchased from TSINGKE, and purified by polyacrylamide gel electrophoresis (PAGE). Various target miRNAs (e.g., miR-1290, miR-141, miR-21) and related match or mismatch probes were mixed and heated at 90 °C for 2 min. The volume ratio of target miRNA to probe was 1:1. Samples were diluted to a final concentration of 0.9  $\mu\text{M}$  hybridization solutions. RNase-free distilled water was used throughout all of the experiments.

### 2.2. Preparation of $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$ Nanobelts

Titanate nanobelts were synthesized via a hydrothermal process in a concentrated NaOH aqueous solution. Briefly, 0.1 g of the P-25 precursor and 9 mg of the  $\text{Ho}_2\text{O}_3$  powders were mixed with 20 mL of a 10 M NaOH aqueous solution, and underwent a hydrothermal treatment at 180 °C in a 25 mL Teflon-lined autoclave for 72 h. The resulting powders were then immersed in a 0.1 M HCl aqueous solution for 24 h and washed thoroughly with de-ionized water. Finally, the collected samples were dried and calcined at 600 °C for 2 h to obtain  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  nanobelts.

### 2.3. Structure Characterization

A Bruker D8 Advance powder X-ray diffractometer was used to investigate  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  nanobelts (a Cu-K $\alpha$  source,  $\lambda = 0.15406$  nm) at room temperature within the range of  $2\theta = 10$  to  $70^\circ$ . Field-emission scanning electron microscopy (Hitachi S-4800) was used to characterize the morphology and size of the synthesized  $\text{Ho}_2\text{O}_3$ - $\text{TiO}_2$  nanobelts.

### 2.4. Electrochemistry Tests

The working electrode is assembled as follows: a glassy carbon electrode (GCE, 3 mm diameter) was polished with 0.05  $\mu\text{m}$   $\text{Al}_2\text{O}_3$  suspensions and rinsed extensively with anhydrous ethanol and de-ionized water, and then electrochemically cleaned in 0.5 M  $\text{H}_2\text{SO}_4$  by cycling potentials between  $-0.3$  and  $+1.8$  V at  $100$   $\text{mV}\cdot\text{s}^{-1}$ . A nafion adhesive and an

ethanol suspension of  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts ( $0.5 \text{ mg mL}^{-1}$ ) were drop-cast onto the cleaned GCE surface. After drying, the resulting electrodes were used as sensing electrodes.

Electrochemical measurements were performed in a three-electrode configuration. The working electrode is the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts-modified electrodes prepared above, a counter electrode (Pt foil with a geometric area of  $1.0 \text{ cm}^2$ ), and an Ag/AgCl/KCl saturated reference electrode. An SWV positive and negative sweep were performed using a CHI660C electrochemical work station, and voltammetric data were acquired. The schematic diagram for experimental procedure is found in the supporting information (see Figure S1).

### 3. Results and Discussion

#### 3.1. Structural Characterization

The synthesized nanobelts were first characterized by XRD measurements. Figure 1 shows the XRD patterns of (a)  $\text{TiO}_2$  and (b)  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts. It can be seen that  $\text{TiO}_2$ - (B) (black dots) and anatase (asterisks) coexist in the  $\text{TiO}_2$  nanobelts (curve a) [20]; and for the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts (curve b), the diffraction peaks appear at  $2\theta = 29, 33, 48$  and  $59$  (denoted by #) that can be assigned to the  $\text{Ho}_2\text{O}_3$  (222), (400), (440) and (622) facets (JCPDS 74-1984) [19]. Moreover, it can be seen that the diffraction peaks of  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  are overall stronger than those of  $\text{TiO}_2$ , indicating the enhanced crystallinity of the former. For example, as compared to curve a, curve b shows a strong and narrow  $\text{TiO}_2$  (004) diffraction peak, suggesting more {001} facets with a high surface energy in the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  sample [21,22].

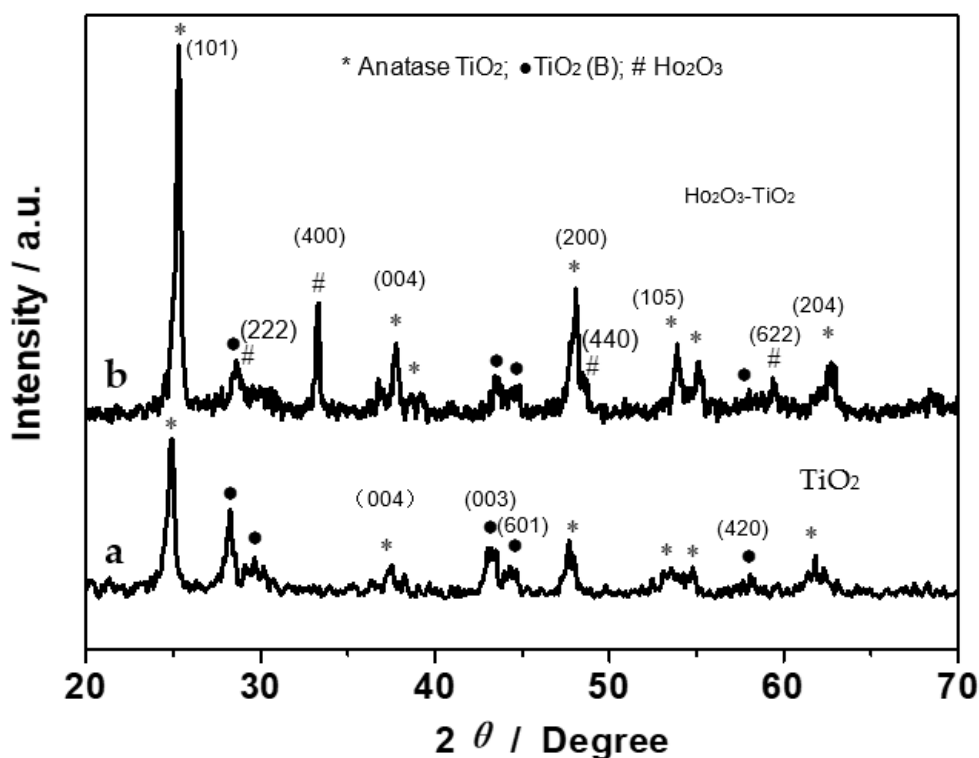
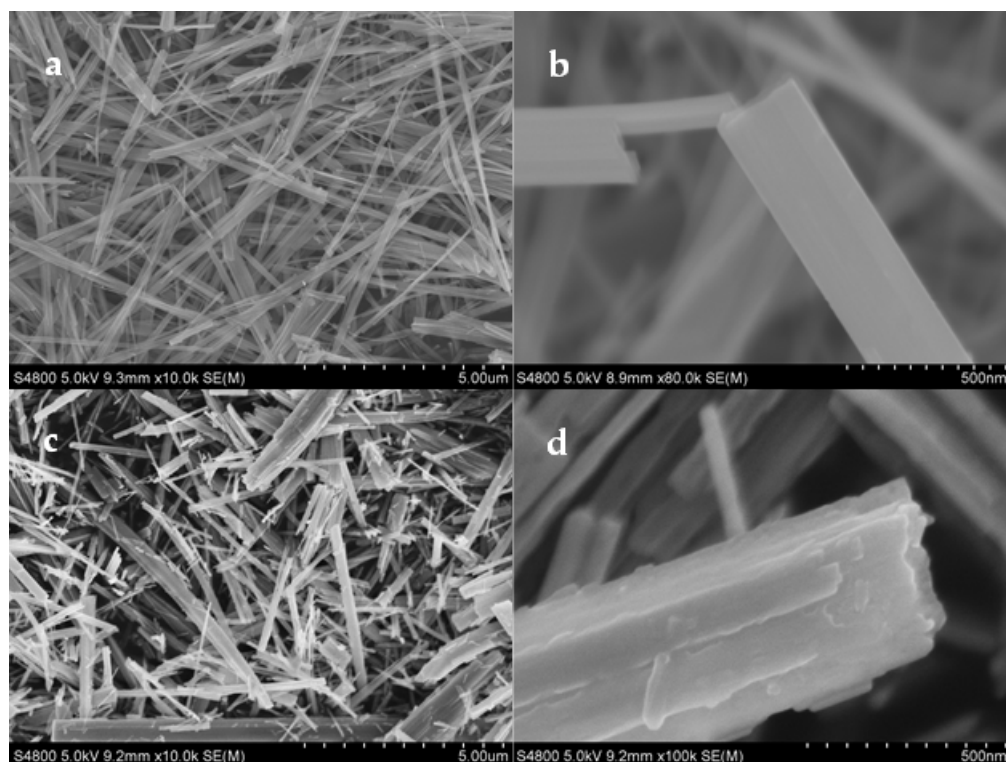


Figure 1. XRD patterns of (a)  $\text{TiO}_2$  and (b)  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts.

FESEM measurements were then carried out to investigate the nanobelts' morphology and microstructure. The morphology of the resulting sample exhibited a drastic change, as  $\text{Ti}^{4+}$  was partly replaced by  $\text{Ho}^{3+}$ . Compared to the  $\text{TiO}_2$  nanobelts (Figure 2a,b), the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts were rougher, thinner, and shorter (Figure 2c,d). Usually,  $\text{TiO}_2$

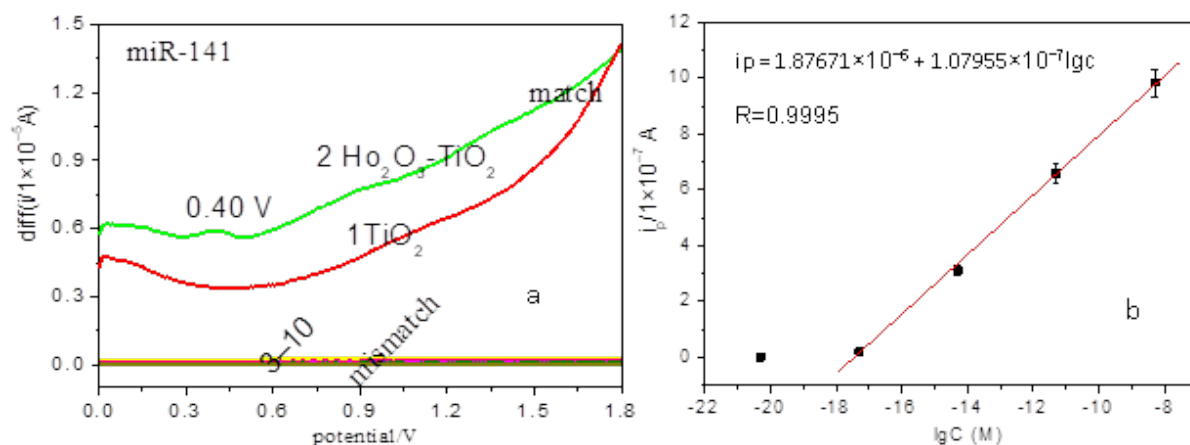
nanobelts grow along the c-axis of the anatase lattice, which makes them lack the {001} facets (Figure 2a,b). As depicted in Figure 1, the TiO<sub>2</sub> sample showed a relatively low and broad (004) peak. On the contrary, Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts displayed a multiple-layer structure and were much thinner than TiO<sub>2</sub> nanobelts, and the surface was roughened, resulting in a significant increase in the effective concentration of the {001} facets (Figure 2c,d). This is consistent with the enhanced (004) diffraction peak in Figure 1b. As Ti<sup>4+</sup> was partly replaced by Ho<sup>3+</sup>, oxygen vacancies might be produced in the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanocrystals, and facilitated the transport of oxygen-containing species as an active material.



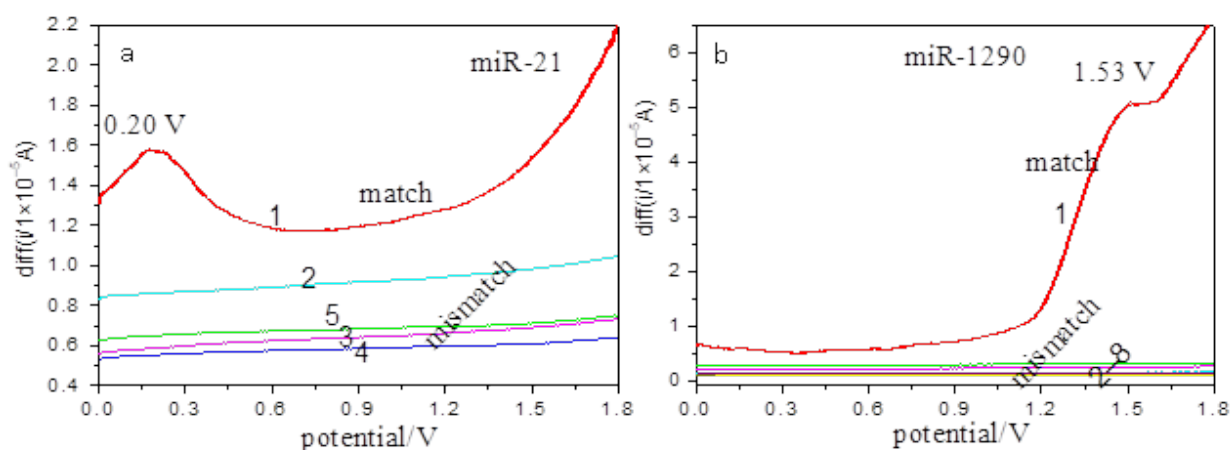
**Figure 2.** Representative SEM micrographs of (a,b) TiO<sub>2</sub> and (c,d) Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts.

### 3.2. Specific and Sensitive Detection of Cancer miRNAs

As is well-known, miR-141, miR-21, and miR-1290 are specific biomarkers of prostate cancer, osteosarcoma, and pancreatic cancer, respectively. The Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts prepared above were then used as a sensing electrode to detect these cancer microRNAs (Figures 3 and 4) by using square-wave voltammetry (SWV). Compared to other electrochemical techniques, SWV possesses the highest sensitivity and specificity in electrochemical pulse techniques.



**Figure 3.** Electrochemical detection of cancer miRNAs. (a) SWV curves for miR-141: (1) match at the  $\text{TiO}_2$  nanobelts modified electrode; match (2) and mismatch (3–10) at the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts modified electrodes; (b) Linear plot of anodic peak current ( $i_{pa}$ ) versus concentration of perfect match miR-141 ranging from 0 to 5 nM at  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts-modified electrodes.



**Figure 4.** Electrochemical detection of cancer miRNAs at the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts-modified electrodes. SWV curves for (a) miR-21: match (1) and mismatch (2–5); (b) miR-1290: match (1) and mismatch (2–8).

Figure 3a shows the SWV positive sweep curves of the perfect match and mismatch for 5 fM prostate cancer miR-141 (related sequences see Table 1) hybridization solutions at the  $\text{TiO}_2$  and  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts modified electrodes. As compared with the featureless response at the  $\text{TiO}_2$  nanobelts-modified electrode (Figure 3a(1)), there is a main anodic peak at +0.4 V and two minor ones at +0.9 V and +1.4 V, (Figure 3a(2)), corresponding to the electrooxidation of perfect match miR-141 at the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts-modified electrodes, which indicates the enhanced electrochemical sensing of the miRNA by the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts. Additionally, for the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts-modified electrodes, no oxidation peak appears for all mismatch solutions within the potential range (Figure 3a3–10), which indicates that  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts can selectively detect prostate cancer miRNA. The better sensing activity of  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts can be explained by results from the XRD (Figure 1b) and FESEM (Figure 2c,d) measurements, where the {001} facets of  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts were evidently increased due to the doping of  $\text{Ho}_2\text{O}_3$ . As is well known, anatase  $\text{TiO}_2$  {001} facets are active for oxygen adsorption with a high activity [23]. As mentioned earlier,  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts consisted of multiple layers and were much thinner than  $\text{TiO}_2$  nanobelts, and had a roughened

surface. This resulted in a significant increase of the effective concentration of the {001} facets and facilitated oxygen adsorption on the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelt surface, leading to the enhanced electrooxidation of miR-141 at the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts electrodes [23]. Note that oxygen adsorption was not favored on stoichiometric anatase TiO<sub>2</sub> {001}, but can be enhanced by the introduction of oxygen vacancy [24]. Compared with the TiO<sub>2</sub> nanobelts, Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts actually contained abundant oxygen vacancies due to the partial replacement of Ti<sup>4+</sup> by Ho<sup>3+</sup> [25], consistent with the enhanced sensing performance.

**Table 1.** Matching and mismatching sequences for prostate cancer miR-141.

No.	miRNA	Sequences
	miR-141	
1,2	Match probe	5'-UAACACUGUCUGGUAAGAUGG-3' 5'-CCATCTTTACCAGACAGTGTTA-3'
	Mismatch probes	
3		5'- <u>A</u> CATCTTTACCAGACAGTGTTA-3'
4		5'- <u>G</u> CATCTTTACCAGACAGTGTTA-3'
5		5'- <u>T</u> CATCTTTACCAGACAGTGTTA-3'
6		5'-CCATCTTTAC <u>C</u> GACAGTGTTA-3'
7		5'-CCATCTTTAC <u>C</u> GACAGTGTTA-3'
8		5'-CCATCTTTAC <u>C</u> GACAGTGTTA-3'
9		5'-CCATCTTTACCAGACAGTGTT <u>C</u> -3'
10		5'-CCATCTTTACCAGACAGTGTT <u>C</u> -3'

Note: mismatched nucleobases are underlined. The serial number in Table 1 is consistent with Figure 3a.

Here, taking miR-141 as an example, we estimated the limit of detection of the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts modified electrode. Different concentrations of perfect match miR-141 were detected at the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts modified electrodes by using SWV measurements (see Figure S2). The variation of the anodic peak current (*i*<sub>pa</sub>) versus concentration of perfect match miR-141 was shown in Figure 3b, which exhibited a linear correlation,  $i_{pa}(A) = 1.87671 \times 10^{-6} + 1.07955 \times 10^{-7} \log(c)$  ( $r^2 = 0.9995$ ) for the perfect match miR-141 within the concentration range of 0 to 5 nM. The *i*<sub>pa</sub> of the blank sample was  $1.2944 \times 10^{-9}$  A at 0.4 V, so the final limit of detection was estimated to be  $4.2658 \times 10^{-18}$  M (or 4.26 aM). The sensitivity is higher than results previously reported for miRNA nano-biosensors [26].

Under the same experimental conditions, detection of 0.9 μM osteosarcoma miR-21 and pancreatic cancer miR-1290 (related sequences in Tables 2 and 3) were also tested with the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts-modified electrodes (Figure 4). From Figure 4, it can be seen that the perfect match miR-21 and miR-1290 showed an oxidation peak at +0.2 V (Figure 4a) and +1.53 V (Figure 4b), respectively. Similarly, no oxidation peaks appear for all mismatch solutions within the same potential range (Figure 4a(2–5),b(2–8)). These results further confirmed that the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts-modified electrodes could selectively detect cancer miRNAs at distinctly different potentials.

**Table 2.** Matching and mismatching sequences for osteosarcoma miR-21.

No.	miRNA	Sequences
	miR-21	
1	Match probe	5'-TAGCTTATCAGACTGATGTTGA-3' 5'-TCAACATCAGTCTGATAAGCTA-3'
	Mismatch probes	
2		5'-TCAACATCAGT <u>G</u> TGATAAGCTA-3'
3		5'-TCAACATCAGT <u>T</u> TGATAAGCTA-3'
4		5'-TCAACATCAGTCTGAT <u>C</u> AGCTA-3'
5		5'-TCAACATCAGTCTGATAAG <u>A</u> T-3'

Note: mismatched nucleobases are underlined. The serial number in Table 2 is consistent with Figure 4a.

**Table 3.** Matching and mismatching sequences for pancreatic cancer miR-1290.

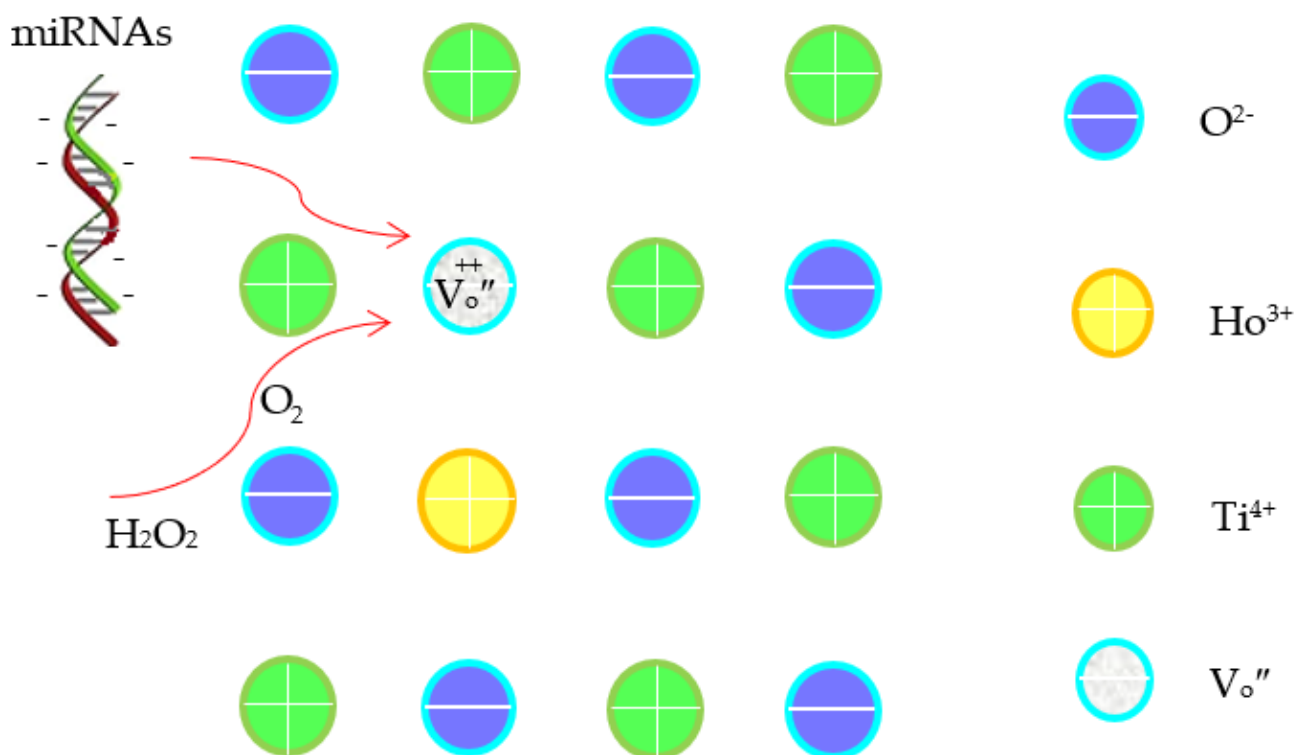
No.	miRNA	Sequences
1	miR-1290	5'-TGGATTTTTGGATCAGGGA-3'
	Match probe	5'-TCCCTGATCCAAAAATCCA-3'
Mismatch probes	2	5'-TCCCTGATC <u>A</u> AAAAATCCA-3'
	3	5'-TCCCTGATC <u>G</u> AAAAATCCA-3'
	4	5'-TCCCTGATC <u>T</u> AAAAATCCA-3'
	5	5'-T <u>C</u> GCTGATCCAAAAATCCA-3'
	6	5'-TCC <u>C</u> GGATCCAAAAATCCA-3'
	7	5'-TCCCTGATCCAA <u>A</u> CATCCA-3'
	8	5'-TCCCTGATCCAAAAAT <u>A</u> CA-3'

Note: mismatched nucleobases are underlined. The serial number in Table 3 is consistent with Figure 4b.

The high selectivity of miRNA detections by Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts can be explained within the context of the molecular structure. MiR-141, miR-21, and miR-1290 exhibit different base sequences and different molecular structures. They can form different hybrid bistrand structures with their respective match probe, which exhibits electrooxidation peaks at different potentials [7]. As shown in Figures 3 and 4, the different cancer miRNA (i.e., miR-141, miR-21, and miR-1290) showed specific voltammetric features, suggesting that cancer miRNAs can be specifically detected via electrochemical potential sensing. Similarly, the perfect match and mismatch of these miRNAs have different hybrid bistrand structures. Compared with the perfect match, the mismatch nucleobases are exposed outside of the hybrid bistrand, resulting in different interactions between the miRNAs hybrid molecules and the electrode surface. Thus, the perfect match and mismatch of the cancer miRNAs exhibited a different electrochemical response, which was amplified by the formation of oxygen vacancy in Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> [24].

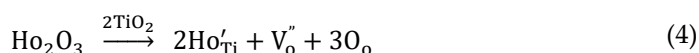
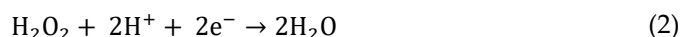
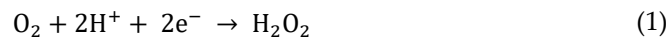
### 3.3. Mechanism of the Electrochemical Detection of Cancer

Within the present potential range, oxygen reduction reaction is inevitable (see Figure S3) [27]. Generally, the electrochemical reaction of O<sub>2</sub> in aqueous media can occur via a pathway involving intermediates such as H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> (Equations (1)–(3)) [27,28]. The strong oxidants can enhance the electro-oxidation of miRNAs on the electrode surface. In comparison with TiO<sub>2</sub> nanobelts, the multiple layers and roughened surface of the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts effectively increased not only the specific surface area but also the concentration of the {001} facets (Figure 2), which further increased the active sites and promoted oxygen adsorption. Additionally, oxygen vacancies were formed in the Ho<sub>2</sub>O<sub>3</sub>-TiO<sub>2</sub> nanobelts since Ti<sup>4+</sup> was partially replaced by Ho<sup>3+</sup> (Equation (4)), and promoted oxygen adsorption on the anatase TiO<sub>2</sub> surface [24] as well as the diffusion of oxygen ions [29]. These collectively improved the surface electrocatalytic properties of the sensing electrodes [30]. Note that oxygen vacancies induced the surface adsorption of oxygen species in different states, i.e., the O-O bond in the superoxide state (O<sub>2</sub><sup>•-</sup>) was oriented parallel to the surface along the {010} facet, while the peroxide state (O<sub>2</sub><sup>2-</sup>) was perpendicular to the surface along the {001} facet, which changed the microenvironment of the sensing electrode surface. Thus, it affects the sensitivity and specificity of the sensing electrodes. Additionally, as the oxygen vacancies are positively charged while miRNAs are negatively charged, the oxygen vacancies can promote the miRNAs adsorption and the oxygen species transfer (Scheme 1) [31,32], leading to enhanced electrochemical detection performance.



**Scheme 1.** The illustration for a mechanism of the electrochemical detection of cancer miRNAs.

Additionally, for the mismatched miRNA molecules, the exposed mismatch bases increase the molecular steric hindrance, and nucleobases have hydrophobicity, all of which affect the mismatched miRNA molecules' adsorption on the  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  electrode surface, resulting in their poor electrochemical sensing response.



#### 4. Conclusions

In this work,  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts were successfully prepared and used as sensing active materials to detect cancer miRNAs. XRD and FESEM measurements indicated an efficient increase of the {001} facets in the nanobelts due to  $\text{Ho}_2\text{O}_3$  doping. Electrochemical measurements showed that  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts could selectively and sensitively detect cancer miRNAs. These results suggest that  $\text{Ho}_2\text{O}_3\text{-TiO}_2$  nanobelts may serve as promising active materials for biosensing applications of early cancer diagnosis, drug screening, and molecular biology research.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/bios12100800/s1>.

**Author Contributions:** J.C. designed and performed experiments, analyzed the data, and wrote the manuscript; X.W. and S.C. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.



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