Supplementary Information

Electrochemical Assay of Superoxide Based on Biomimic Enzyme at High Conductive TiO_2 Nanoneedles: From Principle to Applications in Living Cells

Yang Tian*, Yongping Luo, Qi Rui

Department of Chemistry, Tongji University, Siping Road 1239, Shanghai 200092, P. R. China

1. Experimental details

2. Electrochemical properties of $Mn_2(PO_4)_3$ at different matrixes

3. Electrochemical reaction process of $Mn_2(PO_4)_3$ at Nafion-modified TiO_2 nanoneedles

4. Selectivity of the present $O_2^{\bullet-}$ biosensor

1. Experimental details

Reagents and Materials. Manganese sulfate and potassium phosphate tribasic trihydrate were obtained from Shanghai Meixing Chemical Technology Co. Ltd (Shanghai, China) and Sinopharm Chemical Reagent Co. Ltd (Shanghai, China), respectively, and used without further purification. Other reagents were of analytical grade and used as received. ITO-coated glass plates with a square resistance of ~10 Ω cm^{-2} were obtained from Shenzhen Nanbo Display Technology Co. Ltd. (Shenzhen, China). All the solutions were prepared with Milli-Q water and were deaerated with high purity nitrogen before experiments. All electrochemical experiments were carried out at room temperature.

Fabrication and Modification of TiO_2 Nanoneedles and Mn^{2+}/Nafion/TiO_2 Films. ITO-coated glass plates were thoroughly cleaned by sonication for 30 min in the following solvents successively: soapy water, neat ethanol, 1 M NaOH and water. A TiO_2 nanoneedle film was prepared as follows: an ITO-coated glass plate was coated with a high conductive (~10 Ω cm^{-2}) nano-TiO_2 sol (Ishihara Sangyo Kaisha, FT-2000), and then sintered at 723 K for 1 h. Nafion-modified or Mn^{2+}-Nafion modified TiO_2 films were prepared by spin-coating 1 ml 0.5% (wt) nafion or Mn^{2+}-nafion composites (1 ml 0.5% nafion (wt) mixed with 10 ml 500 mM $MnSO_4$) onto the TiO_2 nanoneedle surface, respectively, and labeled as Nafion/TiO_2 or Mn^{2+}/Nafion/TiO_2 film.

Apparatus and Measurements. A CHI 660 and CHI 832 electrochemical work stations (CH Instruments) were employed in all electrochemical measurements. The reference electrode was a KCl saturated Ag|AgCl electrode, while the auxiliary electrode was a platinum wire. SEM images were taken by a Quanta 200 FEG (FEI Company). The $O_2^{\bullet-}$ solutions were prepared by the addition of aliquots of KO_2 stock solution (N_2-saturated).[1] The concentration of $O_2^{\bullet-}$ was determined by recording the reduction of ferricytochrome *c* spectrophotometrically and using the extinction coefficient (21.1 $mM^{-1}cm^{-1}$) of ferrocytochrome *c* at 550 nm.[2] After a stable background current was obtained under the applied potential conditions, KO_2 was pipetted into the PBS to generate $O_2^{\bullet-}$. The introduction of KO_2 into the electrolyte solution produced a rapid and obvious

increase in the anodic current. Well-defined steady-state current responses were obtained at both 600 and 0 mV, and the currents increased stepwise with successive additions of KO_2. The steady-state currents at 600 and 0 mV were proportional to the rate of $O_2^{\bullet-}$ generation in the examined range. The calibration plots were obtained from these data.

Cell Culture and Detection of $O_2^{\bullet-}$ Released from Living Cells. Chinese hamster normal (HEK 293T) and cancer (CHO) ovary cell lines were obtained from Tongji Hospital (Shanghai, China) and cultured in flasks at 37°C. The medium contained 10% fetal bovine serum (FBS) in RPMI-1640 supplemented with 2 mM L-glutamine, 50 IU mL^{-1} penicilin, and 50 μg mL^{-1} streptomycin. The medium was changed every third day. For cell adhesion, 0.5 mL of cells at a concentration of 2.0×10^5 cells mL^{-1} was directly plated onto the Nafion/TiO_2 film and Mn^{2+}/Nafion/TiO_2 film for the electrochemical experiments. The adhered cells were fixed with 2% glutaraldehyde for 20 min at room temperature. For the detection of $O_2^{\cdot-}$ released from living cells, the geometric surface area of nanostructured TiO_2 film was fabricated to be 2 mm in width and 10 mm in length. Real sample measurements were performed in 100 mM K_3PO_4 containing 100 mM glucose.

2. Electrochemical properties of $Mn_2(PO_4)_3$ at different matrixes

Table S1. Electrochemical Properties of $Mn_2(PO4)_3$ at Different Matrixes

Matrixes	$E^{0'}$ (mV)	k_s (s^{-1})	α_c
Glassy carbon electrode	555.6	0.12	0.42
Cysteine-modified gold electrode	450.1	0.51	0.48
Spherical TiO_2 film	540.3	0.05	0.44
Mn^{2+}/Nafion/TiO_2 film	552.4	7.03	0.52

3. Electrochemical reaction process of $Mn_2(PO_4)_3$ at Nafion/TiO_2 film

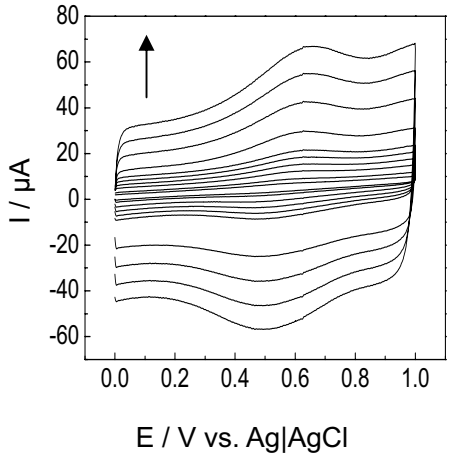

Figure S1. CVs obtained at Mn^{2+}/Nafion/TiO_2 Film in 100 mM K_3PO_4 (pH 7.0) at 10, 20, 50, 100, 200, 300, 400, 500 mV s^{-1}.

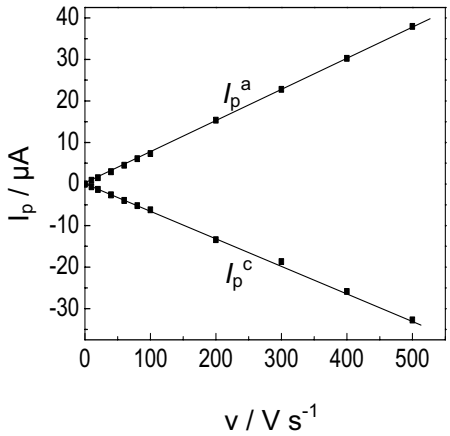

Figure S2. Relationship between peak currents (I_p) and scan rates (v).

Both anodic and cathodic peak currents increase with the increasing potential scan rate (Figure S1), and are proportional to the scan rate (Figure S2), as expected for an electrochemical process with a surface-confined species. According to Laviron's procedure,[3] the relevant kinetic parameters of the electrode reaction, i.e. the rate constant of the electrochemical process (k_s) and cathodic transfer coefficients (α_c), were calculated: k_s = 7.03±0.64 s^{-1}, α_c = 0.52±0.02 at the nanostructured TiO_2 surface.

Electrochemical behaviors of Mn^{2+}/Nafion/TiO_2 Film in 100 mM K_3PO_4 with different pH values were also investigated. As shown in Figure S3, the formal potential ($E^{0'}$) of Mn^{2+}/Nafion/TiO_2 Film decreased with the increasing pH value. In addition, the slope of $E^{0'}$/pH is near 59 mV/pH, indicating that the electrochemical process is one electron – one proton.

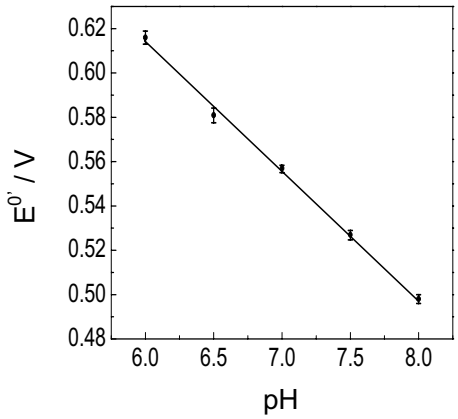

Figure S3. Relationship between the formal potential ($E^{0'}$) of Mn^{2+}/Nafion/TiO_2 Film in 100 mM K_3PO_4 and pH.

4. Selectivity of the present $O_2^{\bullet-}$ biosensor

Table S2. Current responses of Mn^{2+}/Nafion/TiO_2 Film for the estimation of $O_2^{\cdot-}$ against the diverse interferents.

Applied potential V vs. Ag|AgCl	Current / nA												
	$O_2^{\cdot-}$	H_2O_2	Fe^{3+}	Ca^{2+}	Mg^{2}	Na^{+}	K^{+}	Zn^{2+}	UA	AA	DA	Cys	O_2
	1 μM	2.5 μM	5 μM	2.5 μM	5 μM	5 μM	5 μM	5 μM	1μM	1μM	1μM	5 μM	200 μM
0.0	49	0.79	0.86	0.45	0.22	0	0	0	0	0	0	0	0.3
		(1.61)[a]	(1.76)	(0.92)	(0.45)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.61)
0.6	42	0	0.20	0	0	0	0	0	0.22	5.73	10.42	1.94	0
		(0)	(0.48)	(0)	(0)	(0)	(0)	(0)	(0.52)	(13.64)	(24.81)	(4.62)	(0)

a) The values given in the parentheses are percentages with respect to the current for $O_2^{\cdot-}$.

References

[1] J. S. Valentine and . B. Curtis, *J. Am. Chem. Soc.* 1975, **97**, 224.

[2] J. M. McCord and I. Fridovich, *J. Biol. Chem.* 1969, **244**, 6049.

[3] E. Laviron, *J. Electroanal. Chem.* 1979, **101**, 19-28.