Manganese Phosphate Self-assembled Nanoparticle Surface and Its application for Superoxide Anion Detection

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Quantitative analysis of superoxide anion (O₂⁻) has increasing importance considering its potential damages to organism. Herein, a novel Mn-superoxide dismutase (MnSOD) mimics, silica-manganous phosphate (SiO₂-Mn₃(PO₄)₂) nanoparticles, were designed and synthesized by surface self-assembly processes that occur on the surface of silica-phytic acid (SiO₂-PA) nanoparticles. The composite nanoparticles were characterized by fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electronic microscopy (SEM), electron diffraction pattern, energy dispersive spectroscopy (EDS) and elemental mapping. Then the electrochemical measurements of O₂⁻ based on the incorporation of SiO₂-Mn₃(PO₄)₂ onto the surface of electrodes were performed, and some satisfactory results were obtained. This is the first report that manganous phosphate (Mn₃(PO₄)₂) nanoparticles with shape-controlled, but not multilayer sheets, were utilized for O₂⁻ detection. The surface self-assembly technology we proposed will offer the ideal material to construct more types biosensor and catalytic system for its nanosized effect.

Active reactive oxygen species (ROS) containing oxygen atoms are the substances with strong oxidizing ability, which can cause or aggravate cancer, cardiovascular diseases, asthma, cataract, ulcer disease, Alzheimer's disease, Parkinson's disease and other diseases. O₂⁻, the critical important part of the so-called ROS, is implicated in many physiological and pathological processes. Under normal physiological conditions, O₂⁻ maintains the relatively balanced level in vivo. Once the cell produces excessive O₂⁻ in response to external stimulus or pathological changes, it will lead to etiology of aging, cancer, and progressive neurodegenerative diseases such as Parkinson's disease. Thus, real-time analysis and detection of O₂⁻ have great significance. A variety of approaches have been tried to measure O₂⁻ concentration, such as electron spin resonance, spectrophotometry, chemiluminescence, colorimetry, chromatography, and fluorescence. However, these methods cost much and usually occupy too much space. In comparison with other methods, the electrochemical method has recently attracted a great deal of attention owing to its advantages including high sensitivity, low detection limit, simplicity, direct, real-time detection and so on.

Up to date, the commonly used electrochemical enzyme sensors are fabricated by immobilizing superoxide dismutase (SOD) and cytochrome (cyt c) onto the electrode surface. However, the enzymatic O₂⁻ sensors are easily affected by pH and temperature changes, which limit their practical applications due to the poor stability of nature enzyme. Nanozymes, possessing enzymatic activities with nanostructure, have attracted particular attention as emerging natural enzyme mimics, they offer the possibility of lowered cost, improved stability, and excellent recyclability. Meanwhile, bionic concept has gained more and more attention. Mn-superoxide dismutase (MnSOD) mimics, manganous phosphate (Mn₃(PO₄)₂), manganous pyrophosphate (Mn₂P₂O₇) and manganese (II) complexes are usually used to fabricate biosensors for O₂⁻ detection. Cabelli have studied the antioxidant mechanism of aggregated Mn₃(PO₄)₂ particles in organic vivo. Li used DNA as a template to

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produce Mn3(PO4)2 nanosheets and decorated this biomimetic enzyme onto the electrode surface for sensitive in-situ detection of O2·−.25 However, the intrinsic drawbacks of DNA, including high cost, instability, and storage difficulty, may limit their widely applications of electrochemical sensors. Dai also reported the high efficient catalysis of Mn2P2O7, which was used as a SOD mimic for O2·− detection26. There is a serious problem in dealing with the preparation of these reported MnSOD mimics. It is that the conventional synthesized MnSOD mimics that reported in the previous literatures have multilayer sheet structure with uncontrolled shape, thickness and size. This approach will bring resources waste and low catalytic efficiency. We wonder how it is possible to utilize surface self-assembly technology and nanotechnology to construct a more efficient MnSOD mimic for promoting analytical properties.

In this paper, SiO2-Mn3(PO4)2 NPs were synthesized by surface self-assembly processes that occur on the surface of SiO2-phytic acid (SiO2-PA). To the best of our knowledge, there are no reports employing surface coating technique to immobilize Mn3(PO4)2 onto the surface of NPs for O2·− detection. The SiO2-Mn3(PO4)2 NPs have many advantages, like controllable shape with nanoscale, high specific surface area than that of nano-sheet structure, low cost, simple preparation process, non-toxic, and so on. This novel MnSOD mimic we prepared is utilized to fabricate biosensors, and the electrochemical measurements of O2·− based on the incorporation of SiO2-Mn3(PO4)2 onto the electrodes surface are performed.

Results and Discussion
Figure 1 showed the fourier transform infrared (FTIR) spectroscopy of SiO2 NPs (a) and SiO2-PA NPs (b). For curve (a), the appearance of characteristic peak at 1106 cm−1 and 957 cm−1 were attributed to the O-Si-O bonds stretching vibration, indicating that SiO2 NPs were successfully synthesized27. Compared with unmodified SiO2 NPs, the SiO2-PA NPs illustrated three extra peaks at 2928, 1552 and 695 cm−1, which should be attributed to -C-NH2 stretching, symmetric -NH2 stretching, and the bending vibrations of -NH in APTES, respectively28. The results indicated that APTES was successfully modified onto the surface of SiO2 NPs29. More importantly, an adsorption peak at 1092 cm−1 was observed due to the overlap of the characteristic peak of phosphate group (PO43−) and the peak of asymmetric O-Si-O stretching30. The results confirmed that the SiO2 NPs were successfully modified by APTES and PA.
As shown in Fig. 2a, the Zeta potential of SiO$_2$ surface was $-38.5$ mV, which was attributed to many -OH and other oxygen-containing groups that present in the SiO$_2$ NPs surface. When modified with APTES, the Zeta potential of APTES-SiO$_2$ NPs increased to $+22.3$ mV due to the amine groups on the surface of the particles (Fig. 2b). However, the Zeta potential measurements for SiO$_2$-PA NPs (Fig. 2c) showed a negative surface charge that owing to the six phosphate groups of PA. When Mn$^{2+}$ ions in solution were self-assembled onto the surface of SiO$_2$-PA NPs, the zeta potential increased to $-14.1$ mV. The change of Zeta potential indicated that SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs were successfully synthesized by self-assembly technology based on the electrostatic interaction that between Mn$^{2+}$ ions and the phosphate groups$^{31}$.

The TEM and SEM images were also employed to further confirm the formation of SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs. Figure 3A revealed that the spherical SiO$_2$ NPs were obtained with the average particle size of 75 nm. After surface self-assembly of PA and Mn$^{2+}$ sequentially, the two sizes of SiO$_2$-PA NPs and SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs showed a slight increase (Fig. 3B, C), respectively. Furthermore, the electron diffraction pattern displayed an amorphous diffraction pattern of Mn$_3$(PO$_4$)$_2$ that deposited on the surface of silica (see the inset from Fig. 3C). And the corresponding elemental mapping of oxygen (O), silicon (Si), phosphorus (P), and manganese (Mn) from the SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs were indicated in Fig. 3D. The energy dispersive spectroscopy (EDS) of SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs showed that the different atomic percentages were 85.32% (O), 13.10% (Si), 1.47% (P), and 0.11% (Mn), respectively. It can be concluded that Mn$_3$(PO$_4$)$_2$ was firmly coated onto the outer surface of the SiO$_2$-PA NPs. Mn$_3$(PO$_4$)$_2$ layer has little effect on the size growth of SiO$_2$ NPs because it was only monolayer of Mn$_3$(PO$_4$)$_2$ molecular that self-assembled onto the outer surface of SiO$_2$ NPs based on the electrostatic interaction. Here, the stability of Mn$_3$(PO$_4$)$_2$ supported on SiO$_2$ NPs was evaluated by Zeta potential measurement after three weeks of storage. As was shown in Figure S1, the Zeta potential of the SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs had almost no change after three weeks of storage, indicating the Mn$_3$(PO$_4$)$_2$ NPs have long-term stability. SEM images were also used to investigate the surface texture change after Mn$_3$(PO$_4$)$_2$ coating on SiO$_2$ NPs, and the particle size of Mn$_3$(PO$_4$)$_2$.

Figure S2 showed the SEM images of the SiO$_2$ NPs, SiO$_2$-PA NPs and SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs, respectively. It can be observed from the SEM results that the samples with Mn$_3$(PO$_4$)$_2$ coating can retain its original spherical morphology. Meantime, it can also be seen in this figure that particle size of the samples showed a slight increase after Mn$_3$(PO$_4$)$_2$ coating on SiO$_2$ NPs, which was in consistence with the results obtained by TEM images as showed.

**Figure 3.** TEM images of (A) SiO$_2$ NPs, (B) SiO$_2$-PA NPs, (C) SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs, and (D) EDS spectrum and elemental mapping of SiO$_2$-Mn$_3$(PO$_4$)$_2$ NPs.
Figure 4. (A) Schematic illustration of the formation of SiO2-Mn3(PO4)2 NPs: (a) Amino-modified process of SiO2 NPs, (b) Phytic acid modified process of SiO2-NH2 NPs, and (c) Self-assembled of Mn3(PO4)2 on the outer surface of SiO2-PA NPs. (B) Schematic diagram for the construction of the biosensor.

Figure 5. (A) Cyclic voltammograms (CVs) of (a) bare GCE, (b) SiO2-Mn3(PO4)2/GCE, (c) SiO2-Mn3(PO4)2/MWCNTs/GCE, (d) Mn3(PO4)2/MWCNTs/GCE, and (e) MWCNTs/GCE, (B) CVs of SiO2-Mn3(PO4)2/MWCNTs/GCE in the presence (a) and absence (b) of 1.0 μmol L−1 O2− in PBS (pH 7.4), scan rate: 100 mV·s−1.

in Fig. 3. Figure 4A illustrated the synthesis process of SiO2-Mn3(PO4)2 NPs. The formation mechanism of this biomimetic enzyme could be explained as follows: After dropping into MnSO4 solution, PO43− ions, derived from the surface of SiO2-PA NPs, were in combination with Mn2+ ions by electrostatic interaction to form Mn3(PO4)2. When the PO43− ions were consumed, the monolayer of Mn3(PO4)2 molecular was self-assembly on the outer surface of SiO2-PA NPs with controllable morphology. In addition, only aggregated Mn3(PO4)2 particles were observed in the absence of SiO2 NPs with the same reaction conditions (Supplementary Figure S3).

A schematic drawing of the stepwise construction process of modified glassy carbon electrode (GCE) was described in Fig. 4B. The electrochemical properties of the SiO2-Mn3(PO4)2/Multi-walled carbon nanotubes (MWCNTs)/GCE were investigated by cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS)32–35 (Supplementary Figure S4). Figure 5A displayed that all fabrication process of SiO2-Mn3(PO4)2/GCE were carried out by CV in nitrogen saturated phosphate buffered solution (PBS) at a scan rate of 100 mV·s−1. In the working potential range of 0–0.9 V, there was no electrochemical signal can be observed at the bare GCE (curve a). In contrast, the SiO2-Mn3(PO4)2/GCE exhibited a pair of weakly redox peaks (curve b). When the MWCNTs/GCE was modified with SiO2-Mn3(PO4)2 NPs, the oxidation-reduction peaks were more obviously observed (curve c) that due to the excellent electronic conductivity of MWCNTs (Supplementary Figure S5), and the sensitivity of this biosensor was largely improved36. Moreover, the peak currents of SiO2-Mn3(PO4)2/MWCNTs/GCE (curve c) were much larger than that of Mn3(PO4)2/MWCNTs/GCE (curve d).

Figure 5. (A) Cyclic voltammograms (CVs) of (a) bare GCE, (b) SiO2-Mn3(PO4)2/GCE, (c) SiO2-Mn3(PO4)2/MWCNTs/GCE, (d) Mn3(PO4)2/MWCNTs/GCE, and (e) MWCNTs/GCE, (B) CVs of SiO2-Mn3(PO4)2/MWCNTs/GCE in the presence (a) and absence (b) of 1.0 μmol L−1 O2− in PBS (pH 7.4), scan rate: 100 mV·s−1.
Results demonstrated that the electro-catalytic effect of SiO₂-Mn₃(PO₄)₂ NPs was much higher than that of Mn₃(PO₄)₂ aggregated particles. It can be attributed to that the nanosized SiO₂-Mn₃(PO₄)₂ NPs possessed high specific surface area. As a result, it will help improve the catalytic efficiency of O₂⁻ in the electrolyte. When the bare GCE was only modified with MWCNTs, the background current was more clearly observed (curve c).

To study the catalysis effect of the SiO₂-Mn₃(PO₄)₂ NPs, the biosensor in PBS and PBS of containing 1.0 μmol L⁻¹ O₂⁻ were measured by CV, respectively. As shown in Fig. 5B, in the PBS containing of 1.0 μmol L⁻¹ O₂⁻ (curve a), both anodic and cathodic peak currents that corresponding to the redox reaction of in PBS (curve b) clearly increased that can be attributed to the oxidation and reduction of O₂⁻, respectively. According to the previous reports, O₂⁻ was converted into O₂ and H₂O₂ during the disproportionation reaction that O₂⁻ was catalyzed by Mn³⁺ in PBS. In the anodic process, O₂⁻ was oxidized to O₂ by the oxidation effect of MnO₂⁺, while MnO₂⁻ was reduced to Mn²⁺. On the contrary, Mn²⁺ was oxidized to MnO₂⁻ in the cathodic process. As demonstrated above, the SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE can be applied to detect O₂⁻ by measuring the oxidation or reduction currents because of the high efficient catalysis of SiO₂-Mn₃(PO₄)₂. To further prove this proposed mechanism/reaction, X-ray photoelectron spectroscopy (XPS) analysis was carried out to analyze the composition and chemical configuration of the SiO₂-Mn₃(PO₄)₂ NPs before and after electrocatalysis process. More details about the XPS spectra of Mn 2p were presented in Figure S6.

The typical current-time plot of SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE at the applied potential of 0.484 V upon successive additions of O₂⁻ was provided in Fig. 6A. In this experiment, while being stirred, O₂⁻ solution was added once per 50 seconds. With the injection of O₂⁻, the response of this biosensor rapidly achieved 95% of the steady-state current within 2.9 s (Supplementary Figure S7). The SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE showed a wide linear range from 0.03 to 0.21 μM and 0.15 to 3.6 μM with the correlation coefficient of 0.9966 and 0.9959, respectively. The relation of the oxidation peak current vs the concentration of O₂⁻ was linear with a detection limit of 0.0175 μM (S/N = 3). The biosensor exhibited more excellent performance than some O₂⁻ biosensors that reported by the previous papers using different electrode materials, such as SOD, Mn₃(PO₄)₂, and Mn₂P₂O₇ (Supplementary Table 1). The corresponding calibration curves for O₂⁻ were depicted in Fig. 6B. The linear equations were i (μA) = 0.00418 − 0.1358c (μM) and i (μA) = −0.01932 + 0.1263c (μM), respectively. The biosensor was applied to the detection of O₂⁻ and displayed excellent electrochemical behavior.

To verify the applicability of SiO₂-Mn₃(PO₄)₂ NPs in detection of O₂⁻, Xanthine/Xanthine Oxidase (XAN/XOD) was selected to generate O₂⁻ (Supplementary Figure S8). In addition, the response of SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE toward O₂⁻ generated by XAN/XOD was investigated by amperometric measurements. As shown in Figure S9, with successive additions of XAN to the solution, a stepwise increase of the current response was observed.

To evaluate the anti-interference performance of detecting O₂⁻, the biosensor was examined by successive additions of O₂⁻ and interfering substances into a 0.1 M PBS at 0.484 V. Figure 7A indicated that there were obviously current responses of the biosensor during the addition of 1.0 μM O₂⁻, while no obviously current response could be observed with the addition of the interferences. With the sequential addition of 5.0 μM Cys, 5.0 μM DA, 10 μM H₂O₂, 10 μM UA and 10 μM AA, the detection current showed changes of 14%, 2.7%, 8.2%, 5.4% and 4.1% with comparison of 1.0 μM O₂⁻, respectively (Fig. 7B). Figure S10 displayed the amperometric response of the SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE by successive additions of 2.0 μM 18-crown-6 once per 50 seconds. Results demonstrated that this biosensor can eliminate the interference and show an excellent selectivity for detection of O₂⁻. To verify the stability, the biosensor was monitored after being stored for three weeks in a refrigerator. Figure 7C indicated that the current response was no apparent decrease, which was much longer than those obtained for enzyme-based O₂⁻ biosensors. In order to investigate the binding firmness of SiO₂-Mn₃(PO₄)₂ NPs decorated onto the MWCNTs/GCE electrode, the reuse ability of the SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE electrode was tested. Figure S11 displayed the CV curves of the biosensor for 20 cycles, which showed almost overlap curves, indicating the biosensor we prepared had a good cycle stability that can attributed to the good binding state of the SiO₂-Mn₃(PO₄)₂ NPs and the MWCNTs/GCE electrode.
Real-time detection performance of the biomimetic enzyme sensor has also been monitored by detecting \( \text{O}_2^- \) released from the HeLa cells. The amperometric responses of the biosensor were obtained at applied potentials of 0.484 V versus Ag/AgCl in 2 mL 0.1 M PBS (pH 7.4) containing \( 5.0 \times 10^5 \text{ cells\cdot mL}^{-1} \). After the injection of 4 \( \mu \text{g mL}^{-1} \) phorbol 12-myristate 13-acetate (PMA), which was reported to generate \( \text{O}_2^- \) from live cells\(^{45}\), the current gradually increased at SiO\(_2\)-Mn\(_3\)(PO\(_4\))\(_2\)/MWCNTs modified electrode. In this work, PMA was used as a stimulant for the cell to exude \( \text{O}_2^- \)\(^{46,47}\). Figure 7D indicated that the strong current signal (0.01457 \( \mu \text{A} \), curve a) was caused by \( \text{O}_2^- \) released from the HeLa cells, considering that SiO\(_2\)-Mn\(_3\)(PO\(_4\))\(_2\) could selectively decomposes \( \text{O}_2^- \). According to the above linear relationship, the \( \text{O}_2^- \) concentration of 0.0765 \( \mu \text{M} \) was calculated. Thence, the amount of \( \text{O}_2^- \) releasing from per \( 10^5 \) cells was calculated to be 0.153 nmol. Meanwhile, in the absence of the HeLa cells and the presence of the treatment of PMA, no obvious current response can be seen on the screen (curve b). To test the effectiveness of this technology, the biomimetic enzyme sensor has also been used to detect the concentration of \( \text{O}_2^- \) in plasma (Supplementary Figure S12).

**Conclusion**

In this case, the SiO\(_2\)-Mn\(_3\)(PO\(_4\))\(_2\) NPs, synthesized via self-assembly technique and nanotechnology, were applied in a biomimetic enzyme biosensor for the detection of \( \text{O}_2^- \). Results revealed that SiO\(_2\)-Mn\(_3\)(PO\(_4\))\(_2\)/MWCNTs/GCE showed high electrocatalytic activity toward \( \text{O}_2^- \), lower detection limit and wide detection range. Furthermore, the biosensor that assembled under optimal conditions exhibited high selectivity of \( \text{O}_2^- \) in the presence of related interference, such as H\(_2\)O\(_2\), UA, AA, DA and Cys. Meanwhile, the long-term stability and good reproducibility of this biomimetic enzyme biosensor were proved. Compared with the Mn\(_3\)(PO\(_4\))\(_2\) multilayer sheets, the modified GCE of SiO\(_2\)-Mn\(_3\)(PO\(_4\))\(_2\) with high specific surface area exhibited more excellent analytical performance. Consequently, the biomimetic enzyme-free sensor was successfully applied to detecting \( \text{O}_2^- \) that released from live cells, which holds a great promising platform for the reliable monitoring of major diseases in future.

**Methods**

**Materials.** Tetraethyl orthosilicate (TEOS) was purchased from Sinopharm Chemical Reagent Co. Ltd. Cetyltrimethylammonium bromide (CTAB) was obtained from Shanghai Lingfeng Chemical Reagent Co. Ltd. (3-Aminopropyl) triethoxysilane (APTES), phytic acid (PA, 70 wt%) solution and phytic acid sodium salt hydrate...
were received from Aladdin Chemistry Co. Ltd (Shanghai, China). Potassium phosphate tribasic trihydrate (K₂PO₄·3H₂O), manganese sulfate monohydrate (MnSO₄·H₂O) and dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Co. Ltd. Potassium hypoxoide (KO₂) was purchased from Alfa Aesar. Multi-walled carbon nanotubes (MWCNTs) was purchased from Shenzhen Nanotech Port Co. Ltd. Triton X-100, nafion (5 wt% solution in lower aliphatic alcohol), 18-crown-6, phorbol 12-myristate 13-acetate (PMA), dopamine (DA), cysteine (Cys), ascorbic acid (AA) and uric acid (UA) were acquired from Aladdin Sigma-Aldrich Co. (USA). Hydrogen peroxide (H₂O₂, 30%) was received from Beijing Chemical Works (China). Phosphate buffer solution (PBS) was obtained by dissolving 8.0 g NaCl, 0.2 g KCl, 1.44 g NaH₂PO₄ and 0.24 g KH₂PO₄ in 1000 mL double-distilled water.

**Apparatus.** The morphologies of the samples were recorded by transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HITACHI H-7650, Japan). Scanning electron microscope (SEM) images were obtained by a Scanning electron microscope (JSM-6300, Japan). XPS measurements were performed on a Thermo ESCALAB 250 using a monochromatic Al X-ray source (1486.6 eV). All the electrochemicals data were measured by CHI 760D electrochemical workstation (Shanghai Chenhua, China). Fourier transform infrared (FTIR) spectra of the SiO₂ NPs and SiO₂-PA NPs were obtained from a V ARIAN Cary 5000 Fourier transform infrared spectrophotometer (VARIAN, USA). Surface potential of the samples was performed by Zeta potential analyzer (Malvern Instruments ZS90). All experiments were carried out using a three-electrode cell equipped, which consisted of a platinum electrode, saturated calomel electrode (SCE) and working electrode.

**Culture of Cells.** The HeLa cells were cultured in Dulbecco’s Modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units·mL⁻¹ penicillin, and 100 μg·mL⁻¹ streptomycin at 37 °C. Then, the HeLa cells were centrifuged for the electrochemical experiments. Real sample measurements were performed by the addition of 100 μg·mL⁻¹ PMA in PBS containing 50 mM glucose.

**Preparation of SiO₂-Mn₃(PO₄)₂ NPs.** Firstly, SiO₂ NPs were synthesized by the reverse microemulsion method as reported previously by Bagwe. In a typical synthesis, triton X-100 (10.62 g), hexanol (9.6 mL) and cyclohexane (45 mL) were mixed in a 100 mL round-bottomed flask under stirring for 10 min, and then water (2.88 mL) was added to the mixture at room temperature. After being stirred for 0.5 h, NH₃·H₂O (600 μL) and TEOS (1200 μL) were dropped into the above clear solution, respectively. Next, the mixture was allowed to stir for a further 24 h at room temperature. The resulting NPs were collected by centrifugation and washed with alcohol and double-distilled water. Finally, the SiO₂-PA NPs were dispersed in water (10 mL), and then MnSO₄ aqueous solution (10 mL, 12 mM) was injected into the above solution with continues stirring for 30 min at room temperature. Then, the SiO₂-NH₂ NPs were obtained by seeding appropriate amount of APTES. After stirring for 30 min, 120 μL of PA/PA sodium salt hydrate buffer solution (pH = 7) was injected into the above solution with continues stirring for 24 h. The resulting NPs were washed with alcohol and double-distilled water. Finally, the SiO₂-PA NPs were dispersed in water (10 mL), and then MnSO₄ aqueous solution (10 mL, 12 mM) was injected to the round-bottomed flask containing the SiO₂-PA NPs under constant stirring for 1 h at 60 °C. After completion of the reaction, the obtained products were collected by centrifugation, washed with double-distilled water, and dried in a vacuum oven at 60 °C for 24 h.

**Fabrication of SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE.** Firstly, MWCNTs (8.0 μL, 2.5 mg·mL⁻¹) were cast onto the electrode surface and dried at room temperature. After that, SiO₂-Mn₃(PO₄)₂ (8.0 mg·mL⁻¹) and 2.5% Nafion with the volumetric ratio of 1:1 were mixed, and 8.0 μL of above solution was dropped onto the surface of MWCNTs/GCE. After drying in air, the SiO₂-Mn₃(PO₄)₂/MWCNTs/GCE was obtained. During the experimental period, the modified electrodes were stored at 4 °C until use.

**Generation of superoxide anion.** A stable O₂⁻⁻ solution was prepared by dispersing KO₂ to DMSO (containing 18-crown-6). In accordance with the molar absorptivity of O₂⁻⁻ in DMSO, the concentration of O₂⁻⁻ was monitored by recording the absorbance of ferricytochrome c spectrophotometrically at 550 nm. In particular, spectrophotometric measurement of the amount of ferricytochrome c that reduced by O₂⁻⁻ referred to the following reaction: cytochrome c (Fe³⁺) + O₂⁻⁻ = ferrocytochrome c (Fe²⁺) + O₂⁻⁻. The linear relationship of absorbance of ferricytochrome c exhibits a strong absorbance at 550 nm. The linear relationship of the absorbance vs the ferrocytochrome c concentration was depicted in Figure S13. The linear equations were A 550 nm = 0.0044, R = 0.9986. The concentration of O₂⁻⁻ can be calculated by the concentration of the formed ferrocytochrome c according to the above reaction formula.

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Acknowledgements
This work was supported by Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, National Natural Science Foundation of China (21571104, 2015M580446), Natural Science Foundation of and Jiangsu Province of China (BK 20131396), and the Priority Academic Program Development of Jiangsu Higher Education Institution.

Author Contributions
C.M. and M.W. proposed and supervised the project. X.S. and F.W. wrote the main manuscript text and discussed the results and experimental conditions with all other authors. X.S., Q.W. and L.M. designed and set up the experimental setup. X.S., Q.W., W.X. and Y.L. carried out the experiments. X.S., W.X. and S.F. designed and carried out the data analysis. All authors have given approval to the final version of the manuscript.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Shen, X. et al. Manganese Phosphate Self-assembled Nanoparticle Surface and Its application for Superoxide Anion Detection. Sci. Rep. 6, 28989; doi: 10.1038/srep28989 (2016).

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