Supporting Information

ROS scavenging Mn₃O₄ nanozymes for *in vivo* anti-inflammation

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Figure S8. EPR spectra of samples containing DMPO, xanthine, and xanthine oxidase in the absence (a) and presence of 2.5 μg/mL (b) or 5 μg/mL (c) Mn$_3$O$_4$ NPs. (d) EPR spectrum of DMPO only.

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Figure S13. Photograph of a live mouse, showing an inflammatory ear (right) induced by PMA.

Figure S14. Total radiant efficiency of the fluorescence images acquired in live mice after different treatments.

Figure S15. H&E stained images of (A) healthy mouse ear, (B) PMA induced inflammation ear, (C) PMA induced inflammation ear treated with 0.5 μg/kg Mn$_3$O$_4$ NPs, and (D) PMA induced inflammation ear treated with 1.25 μg/kg Mn$_3$O$_4$ NPs.

Figure S16. H&E stained histology sections of different tissues (liver, spleen, and kidney). Mn1 and Mn2 represent low (0.5 μg/kg) and high concentration (1.25 μg/kg) of Mn$_3$O$_4$ NPs, respectively.

Table S1. BET surface area of CeO$_2$ and Mn$_3$O$_4$ nanozymes.
Figure S1. TEM images of (A) Mn$_3$O$_4$ NPs and (B) CeO$_2$ NPs.
Figure S2. Dynamic lighting scattering results of newly-prepared and two-month stored Mn$_3$O$_4$ NPs.
Figure S3. Zeta potential distribution of newly-prepared and two-month stored Mn$_3$O$_4$ NPs.
Figure S4. X-ray photoelectron spectrum of Mn$_3$O$_4$ NPs.
As shown in Figure S5, all of the three catalysts (i.e., Mn$_3$O$_4$ NPs, CeO$_2$ NPs, and natural SOD) exhibited good ‘O$_2^-$’ scavenging activity. However, after pretreatment in 80 °C water bath for 1 h, natural SOD almost lost its activity, while the Mn$_3$O$_4$ NPs and CeO$_2$ NPs still retained high ‘O$_2^-$’ scavenging activity. This result demonstrated that the excellent thermal stability of Mn$_3$O$_4$ and CeO$_2$ nanozymes.

**Figure S5.** ‘O$_2^-$’ elimination efficiency of Mn$_3$O$_4$ NPs, CeO$_2$ NPs, and natural SOD pretreated at 37 °C and 80 °C.
Figure S6. Temperature dependent ‘O₂’ scavenging activities of Mn₃O₄ NPs and natural SOD.
Figure S7. (A) Fluorescent spectra of HE after reaction with xanthine and xanthine oxidase in the presence of Mn$_3$O$_4$ NPs (20 μg/mL) stored for different times. (B) '$O_2^-$ elimination of Mn$_3$O$_4$ NPs after different storage times.
Figure S8. EPR spectra of samples containing DMPO, xanthine, and xanthine oxidase in the absence (a) and presence of 2.5 μg/mL (b) or 5 μg/mL (c) Mn₃O₄ NPs. (d) EPR spectrum of DMPO only.

•O₂⁻ generated from xanthine and xanthine oxidase was trapped by DMPO, which was subsequently detected by EPR (Figure S8a). The characteristic EPR spectrum of DMPO•OOH gradually decreased with the increase of Mn₃O₄ concentration, demonstrating the •O₂⁻ scavenging activity of Mn₃O₄ NPs.
To understand the effect of surface area on the SOD mimicking activity, we investigated the surface area normalized (i.e., specific) SOD mimicking activity of Mn$_3$O$_4$ NPs and CeO$_2$ NPs. As shown in Table S1, the surface area of CeO$_2$ NPs was 166.43 m$^2$/g, which was higher than that of Mn$_3$O$_4$ NPs. Therefore, compared with CeO$_2$ NPs, Mn$_3$O$_4$ NPs showed even better specific SOD mimicking activity than the mass based activity (~2.4-fold enhancement vs ~1.5-fold enhancement) (Figure S9).
As shown in Figure S10, all of the three catalysts (i.e., \( \text{Mn}_3\text{O}_4 \) NPs, \( \text{CeO}_2 \) NPs, and natural SOD) exhibited good \( \text{H}_2\text{O}_2 \) decomposition activity (i.e., catalase mimicking activity). However, after pretreatment in 80 °C water bath for 1 h, natural catalase almost lost its activity, while the \( \text{Mn}_3\text{O}_4 \) NPs and \( \text{CeO}_2 \) NPs still retained high \( \text{H}_2\text{O}_2 \) decomposition activity. This result demonstrated the better thermal stability of \( \text{Mn}_3\text{O}_4 \) nanozymes than natural catalase.
Figure S11. Time evolution of absorbance of $\text{H}_2\text{O}_2$ at 240 nm during the catalytic elimination of $\text{H}_2\text{O}_2$ by Mn$_3$O$_4$ NPs.
Figure S12. EPR spectra of DMPO, Fe$^{2+}$, and H$_2$O$_2$ in the absence (a) and presence of 2.5 μg/mL (b) or 5 μg/mL (c) Mn$_3$O$_4$ NPs. (d) EPR spectrum of DMPO only.

’OH generated from Fe$^{2+}$ and H$_2$O$_2$ was trapped by DMOP, which was subsequently detected by EPR (Figure S12a). The characteristic EPR spectrum of DMPO/’OH gradually decreased with the increase of Mn$_3$O$_4$ concentration, demonstrating the ’OH scavenging activity of Mn$_3$O$_4$ NPs.
**Figure S13.** Photograph of a live mouse, showing an inflammatory ear (right) induced by PMA.
Figure S14. Total radiant efficiency of the fluorescence images acquired in live mice after different treatments.

The total radiant efficiency was calculated by living image 4.5.1 software system. The total radiant efficiency of mice treated with PMA or DCFH-DA only was much lower than those treated with both PMA and DCFH-DA. The radiant efficiency gradually decreased with the increase of Mn$_3$O$_4$ concentration, demonstrating the ROS scavenging activity of Mn$_3$O$_4$ NPs in vivo.
Figure S15. H&E stained images of (A) healthy mouse ear, (B) PMA induced inflammation ear, (C) PMA induced inflammation ear treated with 0.5 \( \mu \text{g/kg} \ Mn_3O_4 \) NPs, and (D) PMA induced inflammation ear treated with 1.25 \( \mu \text{g/kg} \ Mn_3O_4 \) NPs.
Figure S16. H&E stained histology sections of different tissues (liver, spleen, and kidney). Mn1 and Mn2 represent low (0.5 μg/kg) and high concentration (1.25 μg/kg) of Mn₃O₄ NPs, respectively.
Table S1. BET surface area of CeO$_2$ and Mn$_3$O$_4$ nanozymes.

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<thead>
<tr>
<th>Nanozymes</th>
<th>BET surface area (m$^2$/g)</th>
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<tbody>
<tr>
<td>Mn$_3$O$_4$ NPs</td>
<td>105.13</td>
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<tr>
<td>CeO$_2$ NPs</td>
<td>166.43</td>
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