Targeted scavenging of extracellular ROS relieves suppressive immunogenic cell death

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Immunogenic cell death (ICD) and tumour-infiltrating T lymphocytes are severely weakened by elevated reactive oxygen species (ROS) in the tumour microenvironment. It is therefore of critical importance to modulate the level of extracellular ROS for the reversal of immunosuppressive environment. Here, we present a tumour extracellular matrix (ECM) targeting ROS nanoscavenger masked by pH sensitive covalently crosslinked polyethylene glycol. The nanoscavenger anchors on the ECM to sweep away the ROS from tumour microenvironment to relieve the immunosuppressive ICD elicited by specific chemotherapy and prolong the survival of T cells for personalized cancer immunotherapy. In a breast cancer model, elimination of the ROS in tumour microenvironment elicited antitumour immunity and increased infiltration of T lymphocytes, resulting in highly potent antitumour effect. The study highlights a strategy to enhance the efficacy of cancer immunotherapy by scavenging extracellular ROS using advanced nanomaterials.

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cer immunotherapies that stimulate the inherent immuno-

nological systems of the body to recognize, attack, and

eradicate tumour cells have demonstrated varying degrees of

success1–3. Recent studies revealed that immunogenic cell death

(ICD) elicited by specific chemotherapy or radiotherapy makes the
dead cell corpses ‘visible’ to dendritic cells (DCs) that present
antigens to T cells with specific antitumour immune responses,
which then control residual tumour cells6–9. However, ICD induced
immune response can be severely weakened and even abolished
by elevated reactive oxygen species (ROS) in the tumour micro-

environment (TME)10,11. In the meantime, T cells become dis-
funtional after reaching the tumour site12–16. Therefore, how to
modulate the level of extracellular ROS is utterly important to
reverse the immunosuppressive nature of the TME.

ICD provides dying cancer cells with stimuli to elicit immune
responses as a tumour vaccine17–21. Under the potent ICD-inducing
therapies, damage-associated molecular patterns (DAMPs) are
secreted from dying cells or exposed on the outer layer of the cell
membrane to facilitate immune responses. Release of abundant high
mobility group protein B1 (HMGB1) as “fire alarm” signals is es-
tial to induce DC maturation22–24. However, HMGB1 is often
oxidized by ROS in the TME and its stimulatory activity is thus
neutralized25. Although there have been strategies reported to
enhance tumour cell death in an immunogenic way by increasing
the amount of released HMGB1 into the TME, the fate of released
HMGB1 has received limited attention26. Therefore, how to keep
the stimulatory activity of HMGB1 in the TME is a major challenge.
Despite the potential, the antitumour immunity triggered by ICD is
also limited, which is mainly due to the dysfunctional T cells in the
TME. Elevated extracellular ROS controls immune regulation and
reduces the proliferation and antitumour function of T cells26–29.
Therefore, strategies to extend the survival of T cells and recover
immune functions by scavenging ROS in the TME are urgently
needed.

In this study, we prepared a ROS nanoscavenger (T^{\text{ECM-NS}}) modified with ECM targeting peptide. Dual-benzaldehyde ter-
minated polyethylene glycol as a caging polymer was introduced to
construct the crosslinked “stealth” delivery system (PEG-
T^{\text{ECM-NS}}) with pH sensitive imine bonds. This intelligent
nanoscavenger can sweep away the ROS from the TME to relieve
the immunosuppressive ICD elicited by oleanthin (OLE) antici-
ancer drug and prolong the survival of T cells for “personalized”
cancer immunotherapy (Fig. 1). When arriving at the tumour site,
the de-shielding of PEG corona triggered by tumour acidity leads
to the exposure of ECM targeting peptide and anchoring on the
ECM, allowing T^{\text{ECM-NS}} to continuously scavenge extracellular
ROS. Meanwhile, T^{\text{ECM-NS}} is oxidized by ROS, inducing
the disruption of nanoparticles to release OLE. Free OLE is inter-
nalized into tumour cells and induces ICD through the release of
HMGB1 from the dying cells. More importantly, due to the extracellu-
lar ROS neutralization by nanoscavenger, HMGB1 can maintain its stimulatory activity to realize DC activation by dying
or dead cells and antigen processing to T cells with effective
antitumour immune response.

**Results**

**Preparation and characterization of ROS nanoscavenger.**

Poly(propylene sulfide) (PPS) was synthesized via anionic
polymerization of propylene sulfide using 3-mercaptopropionic
acid as an initiator (Supplementary Fig. 1). The chemical
structure and composition of PPS were measured by ^{1}\text{H} NMR,
^{13}\text{C} NMR, and gel permeation chromatography (GPC) (Supple-
mentary Figs. 2–4). Polyetherimide-g-poly(propylene sulfide)
(PEI-PPS) was synthesized via the amidation reaction of PEI
and PPS in the presence of (2-(1H-benzotriazol-1-yl)-1,1,3,3-

tetramethyllumonium hexafluorophosphate/N,N-diisopropyleth-
lamine (Supplementary Fig. 5). The chemical structure of PEI-PPS
was confirmed by ^{1}\text{H} NMR (Supplementary Fig. 6). PEG with
dual aldehyde end groups was obtained by the ester-
ification reaction of PEG and p-formylbenzoic acid in the
presence of dicyclohexylcarbodiimide/4-dimethylaminopyridine
(Supplementary Fig. 7). The structure and composition were
characterized by ^{1}\text{H} NMR, ^{13}\text{C} NMR, and GPC (Supplementary
Figs. 8–10). Collagen targeting peptide (T^{\text{TECM}}) was prepared by
automated solid-phase peptide synthesis at a 100 μmol scale on a
runk amide resin using standard Fmoc-based protocols30 (Supple-
mentary Fig. 11). The structure of peptide was confirmed by high
performance liquid chromatography (HPLC) and liquid
chromatography–mass spectrometry (Supplementary Figs. 12 and
13). Nanoparticle of PEI-PPS was prepared by a dialysis method,
termed as nanoscavenger (NS). Then, tumour collagen targeting
peptide (T^{\text{TECM}}) was conjugated onto the surface of PEI-PPS
nanoparticles through an amidation reaction, termed as T^{\text{ECM-NS}}
(Fig. 2a). The conjugation efficiency is about 90%. The “stealth”
nanoparticles (PEG-T^{\text{ECM-NS}}) were prepared and crosslinked by
the PEGylation of CHO-PEG-CHO via pH sensitive benzoic
imine bond on the surface of T^{\text{ECM-NS}}. The coating efficiency
of PEG on the surface of nanoparticles was about 100% (Supple-
mentary Fig. 14). The morphology of NS, T^{\text{ECM-NS}}, and PEG-
T^{\text{ECM-NS}} were examined using transmission electron microscope
(TEM) (Fig. 2b). TEM result indicated that NS, T^{\text{ECM-NS}}, and
PEG-T^{\text{ECM-NS}} have spherical structures and the average
 diameters were about 100 nm. The size distribution and zeta
potential of NS and T^{\text{ECM-NS}} were evaluated by a laser particle
size analyzer (Fig. 2c, d). As shown in Fig. 2e, the size of nano-
particles remained unchanged after coating PEG on the surface
of T^{\text{ECM-NS}}. However, the zeta potential was decreased with
increasing amount of PEG (Fig. 2f). Because the binary PEG-
T^{\text{ECM-NS}} with T^{\text{ECM-NS}}/PEG mass ratio of 10:2 could efficiently
shield the positive charge of T^{\text{ECM-NS}}, we chose this particular
formula for further experiments. Bovine serum albumin (BSA)
incubation (Supplementary Fig. 15) showed no obvious adsorp-
tion of BSA, indicating the potential high stability of PEG-T^{\text{ECM-NS}}
in blood circulation, due to the protection by PEG caging
copolymer.

Then, the ROS scavenging capacity and collagen binding ability
of PEG-T^{\text{ECM-NS}} were evaluated. The characteristics of OLE
loaded PEG-T^{\text{ECM-NS}} were summarized (Supplementary
Table S1). The accumulated drug release curve of PEG-T^{\text{ECM-NS}}
at pH 7.4 and 6.8 with or without 10 mM H_{2}O_{2} was shown in
Fig. 2g. The drug release of PEG-T^{\text{ECM-NS}} at pH 7.4 with or
without 10 mM H_{2}O_{2} was negligible and less than 25% in 24 h,
indicating that the crosslinked structure of PEG-T^{\text{ECM-NS}} was
relatively stable under neutral condition. The accumulative
amount of OLE from PEG-T^{\text{ECM-NS}} at pH 6.8 in the presence of
10 mM H_{2}O_{2} was 3.21-fold higher than that at pH 7.4 with or
without 10 mM H_{2}O_{2} in 24 h. The release rate of OLE was signi-
cantly enhanced at pH 6.8 with the presence of 10 mM H_{2}O_{2}, under
which the PEG coating on the surface of T^{\text{ECM-NS}} was de-
shielded due to the breakage of pH sensitive imine bonds. Despite
the removal of PEG, T^{\text{ECM-NS}} was able to maintain the intact
nanoparticle structure (Supplementary Figs. 16 and 17). Therefore,
without H_{2}O_{2}, the drug release rate of PEG-T^{\text{ECM-NS}} at pH 6.8
was only about 28% and slightly faster under physiological
condition (about 23%). The significant increased OLE release from
PEG-T^{\text{ECM-NS}} at pH 6.8 with 10 mM H_{2}O_{2} (Fig. 2g) was
attributed to pH-induced de-shielding of PEG and hydrophilic
transition of thioether to sulfoxide, inducing the disassembly of
the nanoparticles. Through the oxidation of thioether residues in
PPS segments to sulfoxides, PEG-T^{\text{ECM-NS}} have the capacity
to scavenge ROS (Supplementary Fig. 18). To assess the ROS
scavenging capability of PEG-TECM-NS, the residual H2O2 concentration after incubation with PEG-TECM-NS was detected using a H2O2 assay kit. Fluorescence at 562 nm was measured as a function of H2O2 concentration in the presence of PEG-TECM-NS pretreated at pH 6.8. There is a sharp decrease in fluorescence at 562 nm in the presence of nanoparticles, suggesting strong H2O2 scavenging activity of PEG-TECM-NS and TECM-NS (Fig. 2h and Supplementary Fig. 19). As a control, we prepared PEI grafted with C18, which was conjugated with TECM on the surface of PEI-C18 nanoparticles (TECM-C18). PEG was also coated on the surface of TECM-C18 (PEG-TECM-C18). Parallel studies showed that PEG-TECM-C18 was incapable of scavenging ROS (Supplementary Fig. 20).

Time-dependent de-shielding of PEG from PEG-TECM-NS was evaluated by 1H NMR. At pH 6.8, after different incubation times, PEG-TECM-NS was centrifuged in a centrifugal dialysis tube (molecular weight cut-off: 30,000 Da). The supernatant was dried and the PEG content was measured by 1H NMR (Fig. 2i). Due to the relatively rapid hydrolysis rate of the imine linker between PEG and TECM-NS, about 85% of PEG was removed from PEG-TECM-NS within 2 h. The binding affinity of PEG-TECM-NS and TECM-NS to collagen was measured by an enzyme-linked immunosorbent assay (ELISA) using biotinylated nanoparticles and substrate coated with rat tail collagen type I (Fig. 2j). TECM-NS with collagen targeting peptides on the surface showed much higher binding affinity with collagen than PEG-TECM-NS. When PEG-TECM-NS was pretreated with pH 6.8 buffer for 2 h to de-shield PEG, the collagen binding affinity was recovered to a similar level with TECM-NS. These results demonstrated that PEGylated PEG-TECM-NS can protect the collagen targeting peptides and improve targeting selectivity. It is expected that the crosslinked PEG on the surface of TECM-NS can shield the collagen targeting peptides during blood circulation. Upon arrival at the tumour site, the rapid de-shielding of PEG corona from the nanoparticles triggered by the TME will lead to the exposure of the collagen targeting peptides to anchor the nanoparticles onto the ECM. Isothermal titration calorimetry (ITC) is a sensitive technique that measures the heat of reaction of two aqueous solutions when one is titrated against the other31–35. As shown in Fig. 2k, each peak represents the enthalpy change caused by the peptide–collagen interactions. The exothermicity of the interaction of the peptide during titration with collagen is very high, indicating the strong binding ability. Furthermore, we also evaluated the selective collagen binding properties of PEG-TECM-NS and TECM-NS in the heterogeneous environment of 4T1 murine mammary tumour. After 4T1 tumour pieces (1 cm3) were incubated with TECM-NS and PEG-TECM-NS at pH 7.4 and pH 6.8, respectively, confocal laser scanning microscopy (CLSM) images of the sectioned slices from the 4T1 tumour were examined. The complete overlap of TECM-NS or PEG-TECM-NS signal at pH 6.8 with anti-collagen antibody further confirmed the collagen specificity of TECM-NS (Fig. 2l and Supplementary Fig. 21).

Investigation of PEG-TECM-NS/OLE-induced ICD and in vitro anticancer efficacy. As mentioned in the above experiments, PEG-TECM-NS with crosslinked structure has high stability during blood circulation. Upon arrival at the tumour site, the de-shielding of PEG corona from the nanoparticles triggered by tumour acidity leads to the exposure of ECM targeting peptide. Then, TECM-NS are anchored on the ECM and retained in the TME without penetration through interstitial space and internalization into tumour cells. The hydrophobic PSS block is oxidized to become hydrophilic and induces the disruption of nanoparticles to gradually release OLE. Free OLE enters tumour cells and induces ICD through the released HMGB1 from the dying cells. To mimic the delivery process in the TME, PEG-TECM-NS, or PEG-TECM-NS/OLE was firstly incubated at pH 6.8 with 100 µM H2O2 for about 12 h and then centrifuged to collect the supernatant. The supernatant containing OLE was used in the following experiments. ICD of tumour cells is characterized by inducing extracellular release of HMGB1 as “find me” signal and

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**Fig. 1 Extracellular ROS nanoscavenger reverses immunosuppression.** Schematic illustration of tumour extracellular matrix (ECM) targeted ROS nanoscavenger masked by pH sensitive covalently crosslinked polyethylene glycol. The nanoscavenger anchored on the ECM sweeps away the ROS from tumour microenvironment to relieve the immunosuppressive ICD elicited by specific chemotherapy and prolong the survival of T cells for personalized cancer immunotherapy.
cell surface expression of calreticulin (CRT) as “eat me” signal. Therefore, the ability of PEG-TECM-NS/OLE to induce ICD was determined by examining the HMGB1 release and CRT exposure by immunofluorescence staining. As shown in Fig. 3a, b, pretreatment of PEG-TECM-NS/OLE at pH 6.8 with 100 µM H2O2 induced more extracellular release of HMGB1 than that of PEG-TECM-NS/OLE without pretreatment. Meanwhile, pretreatment of PEG-TECM-NS/OLE at pH 6.8 with 100 µM H2O2 had...
the similar ability to induce extracellular release of HMGB1 to that of free OLE. These results indicated that PEGylated TECM-NS/OLE with crosslinked structure could restrain drug leakage in physiological environment. The combination of the pH sensitive cleavage of the benzoic imine with de-shieldable PEG and ROS mediated hydrophilic transition of thioether induced disruption of nanoparticles could rapidly trigger drug release. Furthermore, HMGB1 release also confirmed that free OLE and PEG-TECM-NS/OLE could efficiently induce immunogenicity of the tumour cells. Released HMGB1 in supernatant of 4T1 cells was measured by ELISA (Fig. 3c). Western blot was also used to evaluate the expression of HMGB1 (Fig. 3d, e). The significantly reduced...
expression of HMGB1 in intracellular 4T1 cells treated with free OLE and PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2 indicated that considerable amount of HMGB1 was released to extracellular environment. Extracellularly secreted and intracellularly distributed ATP from tumour cells were measured (Fig. 3f). ATP secretion was studied to further verify the ability of PEG-TECM-NS/OLE to induce ICD. We found the intracellular ATP in the control group was significantly higher than that of PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2. ATP secretion in the group of PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2 was 6.93-fold higher than that of that control group (Fig. 3f). These results suggest that ICD was elicited by OLE and PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2. CLSM and flow cytometry results showed obvious CRT translocation to the cell membrane of 4T1 cells treated with free OLE and PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2 (Fig. 3g–j).

To further determine the therapy efficiency of PEG-TECM-NS/OLE as a drug delivery system, the in vitro cytotoxicity of free OLE and PEG-TECM-NS/OLE were evaluated and compared. As shown in Fig. 3k, l, the percentage of late apoptotic cells (Annexin V-FITC and 7-AAD double stained) was 24.7% when treated with PEG-TECM-NS/OLE pretreated with pH 6.8. Consistent with the results, the IC50 values of PEG-TECM-NS/OLE in 4T1 cells with or without pH 6.8 buffer treatment were 0.826 and 4.75 µM L-1, respectively (Fig. 3m, n). In the live cell assay, few live cells were observed in the group of PEG-TECM-NS/OLE compared with that of PEG-TECM-NS/OLE (Fig. 3o, p). In mice, chemotherapy-driven ICD was related to endoplasmic reticulum stress. The swelled endoplasmic reticulum morphology was observed in the group of free OLE and PEG-TECM-NS/OLE pretreated with pH 6.8 buffer, confirming ICD was elicited by OLE and PEG-TECM-NS/OLE (Fig. 3q).

PEG-TECM-NS activated immunogenicity of tumour cells. We further evaluated the efficacy of PEG-TECM-NS/OLE-induced immunogenicity of the tumour cells and the ability to turn the tumour cells into antigen-presenting cells (APC) via ICD. 4T1 tumour cells were treated with free OLE, PEG-TECM-NS/OLE, and PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2 for 12 h. Then, Bone marrow dendritic cells (BMDCs) were co-cultured with pretreated 4T1 tumour cells for another 24 h. The frequency of CD80+CD86+ mature BMDCs after co-culture with 4T1 tumour cells pretreated with free OLE was significantly higher that of the control group, indicating that ICD from dying 4T1 cells elicited by OLE can induce DC maturation and immune response (Fig. 4a, b, and Supplementary Fig. 2). The group of PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2 showed strong immune response with 36.9% CD80+CD86+ BMDCs. These results also indicated that DAMPs secreted from dying cells or exposed on the outer layer of the cell membrane can maintain their activity to facilitate immune responses in normal environment. Meanwhile, interleukin 12 (IL-12p40) and tumour necrosis factor α (TNF-α) as indicators of DC activation elicited by ICD from dying tumour cells were determined by ELISA (Fig. 4c). The result indicated that the secretion levels of IL-12p40 and TNF-α from BMDCs in groups of free OLE and pretreated PEG-TECM-NS/OLE (pH 6.8, 100 µM H2O2) were higher than those of the control group, confirming that OLE could induce strong antitumour immunity. PEG-TECM-NS/OLE with pretreatment induced higher level of IL-12p40 and TNF-α than PEG-TECM-NS/OLE without pretreatment, suggesting that the release of OLE was significantly accelerated at acid environment in the presence of H2O2 due to the pH-induced PEG de-shielding and ROS induced hydrophilic transition of thioether, resulting in the disruption of nanoparticles. Mice were immunized intravenously with the tumour lysate three times. Lymph nodes were then harvested to measure the percentages of CD80+CD86+ DCs. The results verified that PEG-TECM-NS/OLE pretreated with pH 6.8 buffer and 100 µM H2O2 could induce the maturation of DCs (Fig. 4d). Then, we evaluated whether ROS could neutralize the stimulatory activity of DAMPs. 4T1 tumour cells were treated with free OLE, PEG-TECM-NS/OLE, and PEG-TECM-NS/OLE pretreated at pH 6.8 with 100 µM H2O2 for 12 h. Then, 100 µM H2O2 was added into the medium and 4T1 tumour cells were continually cultured for 4 h. Thereafter, BMDCs were co-cultured with pretreated 4T1 tumour cells for another 24 h. The percentage of CD80+CD86+ mature BMDCs was very low in the group of OLE, indicating that the HMGB1 released from dying cells was oxidized by H2O2 and its stimulatory activity was neutralized (Fig. 4e, f). The secretion levels of IL-12p40 and TNF-α of the group of OLE were very low (Fig. 4g). In addition, the ROS nanocanvenger TECM-NS/OLE involved the BMDCs/4T1 co-culture system obviously recovered the immune responses. T-cell proliferation was used to evaluate whether H2O2 influenced the immune responses of T cells. Splenocytes were isolated and stimulated with concanavalin A (ConA) and then incubated with or without 100 µM H2O2 or PEG-TECM-NS. T-cell proliferation was measured by carboxyfluorescein (CFSE) dilution (Fig. 4h, i). The proliferation of CD8+ T cells was significantly inhibited after H2O2 treatment. In the group of PEG-TECM-NS (pH 6.8, 100 µM...
H$_2$O$_2$, the suppressive effect of H$_2$O$_2$ on T-cell proliferation was partially abrogated, showing that PEG-TECM-NS can scavenge extracellular ROS to extend the survival of T cells and recover immune responses. We also found H$_2$O$_2$ concentration dependent inhibition of T-cell proliferation (Supplementary Figs. 23 and 24).

**In vivo antitumour activity of PEG-TECM-NS/OLE.** The in vivo distribution of Cy5.5-labelled PEG-TECM-NS in 4T1 tumour-bearing mice was evaluated. As shown in Fig. 5a, b, PEG-TECM-NS with crosslinked structure exhibited higher tumour fluorescence signal, compared with the nanoparticles without crosslinker. We also quantified the concentrations of OLE in the blood
The ability of PEG-T\textsuperscript{ECM} NS/OLE to inhibit tumour growth was evaluated in 4T1 tumour-bearing BALB/C mice. PEG-T\textsuperscript{ECM} C\textsubscript{18}/OLE without ROS scavenging ability as the control group was also evaluated. As shown in Fig. 5g, h, free OLE showed a substantial reduction in relative body weight (%), indicating a potential adverse effect. In contrast, PEG-T\textsuperscript{ECM} NS/OLE demonstrated a more controlled reduction in body weight, highlighting the significance of prolonged blood circulation and enhanced tumour accumulation.

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moderate reduction of tumour growth by 25% as compared to the PBS group. PEG-TECM-NS/OLE showed better antitumour effect than free OLE as evidenced by 55% reduction in tumour growth compared with the PBS group. Notably, PEG-TECM-NS/OLE achieved 92% reduction in the tumour volume. As shown in Fig. 5i, no significant change in the body weight of mice treated with PEG-TECM-NS/OLE was observed during treatment, verifying the biocompatibility and safety of PEG-TECM-NS/OLE. The survival time mice in each group correlated well with the tumour inhibition result (Fig. 5i). Dying tumour cells generated HMGB1 through PEG-TECM-NS/OLE-induced ICD. The immunostimulatory activity of HMGB1 was activated because the ROS in the TME was effectively cleared by PEG-TECM-NS/OLE nanoscavenger. The immunostimulatory activity of HMGB1 elicited by PEG-TECM-NS/OLE was evaluated by immunofluorescence assay (Fig. 5k). PEG-TECM-NS/OLE treatment with ROS scavenging ability can evoke large amount of HMGB1 in the extracellular environment compared with PEG-TECM-C18/OLE without ROS scavenging ability. CRT exposure induced by PEG-TECM-NS/OLE was much higher than that of the other groups, suggesting that PEG-TECM-NS/OLE realized effective ICD. Modulating the level of extracellular ROS in the TME has been reported to extend survival of T cells\(^{13,15,29,39}\). PEG-TECM-NS/OLE treatment increased CD8\(^+\) T-cell permeation in 4T1 mouse model as compared to PEG-TECM-C18/OLE. As shown in Fig. 5k, the immunofluorescence staining results indicated that PEG-TECM-NS/OLE significantly increased the infiltration of CD8\(^+\) T cells in the tumour region. To evaluate the antitumour immunity, peripheral blood serum was harvested from 4T1 tumour mice with different treatments on day 7 (after the first treatment) and analysed by ELISA. As shown in Fig. 5l, the production of IL-12p40, TNF-\(\alpha\), and interferon-gamma (IFN-\(\gamma\)) were increased in the mice treated with PEG-TECM-NS/OLE. The result indicated that PEG-TECM-NS/OLE can elicit effective ICD. As shown in Fig. 5m, the expression of HMGB1 and CRT protein in cells treated with PEG-TECM-NS/OLE was obviously elevated, which agreed well with the result in Fig. 5k. Compared with other groups, administration of PEG-TECM-NS/OLE markedly reduced the number of Ki67 proliferating tumour cells and increased the number of DUPT nick-end labelling (TUNEL)-positive tumour cells (Fig. 5n). Moreover, hematoxylin and eosin (H&E) staining of the tumour tissue of mice treated with PEG-TECM-NS/OLE showed extensive tumour cell death. No observable damage to normal tissues and major organs was found, suggesting the biocompatibility of PEG-TECM-NS/OLE (Supplementary Fig. 26). As shown in Fig. 5o–q and Supplementary Fig. 27, compared with the control groups, the increased number of IFN-\(\gamma\) expressing CD8\(^+\) T cells and decreased number of Foxp3\(^+\)CD4\(^+\) T cells in the group of PEG-TECM-NS/OLE further elicited powerful antitumour T-cell response. The t-SNE analysis of tumour tissues from mice after different treatments indicated increased IFN-\(\gamma\)CD8\(^+\)T cells, CD8\(^+\) T cells, and T memory cells infiltration into tumours treated PEG-TECM-NS/OLE (Fig. 5r). Then, we established an orthotopic colorectal cancer model with CT26 tumour cells. Intraperitoneal injection of L-012 into orthotopic colorectal tumour mice allowed to detect ROS scavenging by nanoparticles at the tumour site. PEG-TECM-NS treatment had reduced bioluminescence signal compared to PEG-TECM-NS without ROS scavenging ability (Fig. 6a). Quantification of bioluminescence signals indicated the ROS levels in the group of PEG-TECM-NS without ROS scavenging ability was 3.41- and 2.52-fold higher than those of PEG-TECM-NS at 24 and 72 h, respectively (Fig. 6b). We investigated the tumour inhibition of PEG-TECM-NS/OLE in orthotopic CT26 tumour mice. As shown in Fig. 6c, PEG-TECM-NS/OLE showed 91.9% reduction in the colon tumour. PEG-TECM-C18/OLE without ROS scavenging ability achieved 68.8% reduction. As shown in Fig. 6d, no significant change in the body weight of mice treated with PEG-TECM-NS/OLE was observed during treatment, verifying the biocompatibility and safety of PEG-TECM-NS/OLE. We performed immunofluorescence analysis to study the expression of HMGB1, CRT, and CD8\(^+\) T cells in the tumour tissues after different treatments. We observed detectable increase of HMGB1 and CRT in the tumour from the group of PEG-TECM-NS/OLE, demonstrating that ICD was efficiently elicited (Fig. 6e). PEG-TECM-NS/OLE treatment increased CD8\(^+\) T-cell permeation as compared to PEG-TECM-C18/OLE. The expression levels of HMGB1 and CRT protein in orthotopic CT26 tumour mice treated with the PEG-TECM-NS/OLE were obviously elevated, which agreed well with the immunofluorescence analysis results (Fig. 6f). Caspase-3 and H&E staining of the tumour tissue of mice treated with PEG-TECM-NS/OLE showed extensive tumour cell death (Fig. 6g). The levels of cytokine secretion in serum were measured by ELISA to monitor the nanoscavenger induced immune response. PEG-TECM-NS/OLE group displayed a significant increase in IL-12p40, IFN-\(\gamma\), and TNF-\(\alpha\) expressions in the serum, which confirmed that nanoscavenger effectively induced ICD and improved the immune responses (Fig. 6h). The proportion of CD8\(^+\) T cells in...
the tumours of mice treated with PEG-TECM-NS/OLE showed an obviously increase over PEG-TECM-C18/OLE without ROS scavenging ability (Fig. 6i). Furthermore, PEG-TECM-NS/OLE induced a higher proportion of IFN-γ+CD8+ T cells in spleens than that of PEG-TECM-C18/OLE (Fig. 6j, k). t-SNE analysis of tumour tissues from mice after different treatments indicated increased IFN-γ+CD8+ T cells, CD8+ T cells and T memory cells infiltration into tumours treated PEG-TECM-NS/OLE (Fig. 6l).

Extracellular ROS abolishes immune response elicited by ICD and functionally suppresses T cells activation. Therefore, we employed a nanoscavenger to sweep away the extracellular ROS at the tumour site to enhance immunotherapy by relieving the immunosuppressive
ICD and increasing infiltration of CD8+ T cells. Overall, these results suggest that scavenging extracellular ROS is a promising strategy to increase the efficacy of cancer immunotherapy.

**Methods**

**Preparation and characterization of nanoscavengers.** PEI-PPS was prepared by a solvent displacement method. Typically, the solution of dimethyl sulfoxide (DMSO) containing 10 mg PEI-PPS polymer was added dropwise to water. The mixture was dialyzed to remove DMSO at room temperature. Then TECM, EDC, and NHS were added to PEI-PPS nanoparticle solution to obtain TECM-modified nanoparticles (TECM-NS). After stirring for 24 h, the mixture was dialyzed. Then the aqueous solution of CHO-PEG-CHO was added to TECM-NS solution to coat PEG on the surface of TECM-NS through benzonic acid.

**ELISA protocol to determine collagen binding constant.** Collagen-coated plates were prepared by adding 50 µL of a 1:1000 dilution of rat tail collagen type I (1.78 mg/mL in 20 mM acetic acid) in TBS (50 mM Tris-HCl, 150 mM NaCl, pH=7.4) to rows A-D on a 96-well plate (4 plates, one per construct). The plates were incubated overnight at 4 °C on a rotating table, then inverted, emptied, and washed with TBS (3 × 300 µL). All the wells (i.e. rows A–H) were then incubated with 100 µL of TBS containing 5% BSA for 2 h at 37 °C, and subsequently washed with 3 × 300 µL of TBS containing 0.1% Tween-20. The plates were incubated with PEI-PPS-TECM-NS for 3 h. The fluorescence was measured.

**Cell apoptosis detection.** 4T1 Cells were seeded in six well plates. The supernatant of PEI-PPS-TECM-NS pretreated with pH 6.8 buffer and 100 µM H2O2, after centrifugation was added and incubated with cells. Then 4T1 cells were stained with Annexin V-FITC (5 µL for one sample)/7-AAD (5 µL for one sample) (FITC Annexin V Apoptosis Detection Kit with 7-AAD, Biologend, Catalog number 64922) for 30 min at room temperature and then added 500 µL binding buffer to analyze by flow cytometry.

**Animal model.** All animal experiments were performed under a National Institutes of Health Animal Care and Use Committee (NIHACUC) approved protocol. BALB/C mice (Harlan, Indianapolis, IN) were subcutaneously implanted with 1 × 106 4T1 cells. 4T1 cells (ATCC) were authenticated for mycoplasma.

**BMD culture and stimulation.** BMDCs were isolated from the femur of BALB/C mice. The bone marrow cells were harvested and treated with ACK lysis buffer for 5 min. The cells were washed twice with PBS. The bone marrow was cultured in medium with GM-CSF (10 ng/mL) and IL-4 (10 ng/mL) at 37 °C for 7 days to acquire immature DC. 4T1 cells were pretreated with free OLE, PEG-TECM-NS/OLE, and PEG-TECM-NS/OLE pretreated with pH 6.8 buffer and 100 µM H2O2. Then, immature BMDCs (1 × 106) were co-cultured with 4T1 cells (1 × 105) for 24 h. After various treatments, DCs stained with antibodies (anti-CD11c-APC, anti-CD80-FITC, and anti-CD86-PE) for 30 min at 4 °C and measured by a flow cytometer. Anti-CD11c-APC-Cy7, anti-CD80-FITC, and anti-CD86-PE were diluted according to the manufacturer’s direction (1:500).

**T-cell proliferation assay.** Splenocytes were isolated and incubated for 4 h. We collected the cells and labelled with CFSE (1 mM) for 5 min. Then, 0.6 mg mL⁻¹ concanavalin A (ConA) and H2O2 were added. After 3 days, the CFSE signal of gated splenocytes was analyzed by a flow cytometer.

**Tumour-infiltrating lymphocytes analysis.** The harvested tumours were explanted and cut into small pieces. Then, the small pieces were immersed in 5 mL collagenase IV (1 mg mL⁻¹) with 0.2 mg mL⁻¹ DNase I for 1 h at 37 °C. Suspensions were filtered and the single cells were stained with fluorescently labelled antibodies (CD4+CD25+Foxp3+ Tregs, CD4+CD8+ T cells, CD8+IFN-γ+ T cells, CD8+CD4+CD62L+ memory T cells). Anti-CD4-PE-Cy5, anti-CD8-PE-Cy7, and anti-CD25-Alexa Fluor 790, anti-CD62L-APC, anti-CD44-PerCP, anti-Foxp3-PE, and anti-IFN-γ-PercP/Cy5.5 were diluted according to the manufacturer’s direction (1:500).

**In vivo PET imaging.** Firstly, we prepared DFO modified PEG-TECM-NS with crosslinked structure (DFO-PEG-TECM-NS). As a control, we also prepared N-PEG-TECM-NS without crosslinked structure DFO-N-PEG-TECM-NS, Zr89-DFO-PEG-TECM-NS, and Zr89-DFO-N-PEG-TECM-NS were intravenously injected into the 4T1 tumour-bearing mice. One intravenous small-animal PET scanner (Siemens, Erlangen, Germany) was used to acquire whole-body PET images at predetermined time points after injection.

**In vivo antitumour therapy.** BALB/C mice were inoculated with 4T1 tumour cells. The mice were randomly grouped and treated with PBS, free OLE, PEG-TECM-C18/OEL, and PEG-TECM-NS/OLE (OLE 1 mg kg⁻¹ for a total dose). Then the tumour volume was calculated using the formula: tumour volume (mm³) = (length × width)² × 1/2.

**Orthotopic CT26 tumour model establishment.** Mice were anesthetized with isoflurane. A small incision was made to exteriorize the caecum. CT26 cells suspension (1.0 × 106) was injected into the caecum wall. Then the incision was sutured. The mice were randomly grouped and treated with PBS, free OLE, PEG-TECM-C18/OEL, and PEG-TECM-NS/OLE (total OLE dose: 1 mg kg⁻¹ for a total dose). Then the tumour volume was calculated using the formula: tumour volume (mm³) = (length × width)² × 1/2.

**Data availability**

The experimental data that support the findings in the current study are available for research purposes within the paper and its Supplementary Information from the corresponding authors on reasonable request. Source data are provided with this paper.

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Author contributions

H.D. and X.C. conceived and designed the research. H.D. and Z.Z. performed the in vivo experiments and analysed the data. H.D., J.Y. and W.Y. performed the in vitro experiments and analysed the data. H.D., H.D., and X.C. co-wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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