The Double-edged Sword Effect of Macrophage Targeting Delivery System in Different Macrophage Subsets Related Diseases

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Research

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Abstract

Monocyte/macrophage targeting drug delivery system (MTDS) has been highly focused as an emerging routine for delivering drugs to various macrophage related diseases. However, the distinguishing ability towards different macrophage related diseases of these systems and the impact of them on macrophage function and disease progression have not been systematically revealed, which is significantly important for active targeting therapeutic or diagnostic strategies. Herein, taking dextran modified polystyrene nanoparticles (DEX-PS) as example, we demonstrate that modification by dextran can specifically enhance the recognition of nanoparticles (NPs) by M2 macrophages in vitro, however, which is obstructed by monocytes in peripheral blood proved by in vivo assays. DEX-PS is not only targeted distributed in tumor, a M2 macrophage related disease, but also highly distributed in M1 macrophages related disease, acute peritonitis. Additionally, DEX-PS play a double-edged role in these two different diseases by reeducating macrophages to pro-inflammatory phenotype. These results suggest that MTDS, even those designed based on the different expression of receptor on different macrophages subtypes, lacks distinguishing ability for different macrophage subsets related diseases in vivo. In addition to the potential impact of these carrier materials on the function of macrophages, in the study of MTDS, great attention should be paid to the distribution of nanoparticles in non-target diseases and the impact on its disease process.

Background

Inspired by the recruitment of monocytes/macrophages in various diseases, tremendous macrophage/monocytes targeted delivery systems (MTDSs) modified by targeting motifs have been engineered and applied in various macrophage related diseases, such as atherosclerosis [1–4], inflammation [5–7], and especially cancer [8–10]. This widespread availability of MTDSs in various diseases is not only an advantage, but also a potential risk. For example, tumor patients often have a variety of inflammation related complications [11–14]. In this case, MTDSs not only targeted deliver drugs to tumor local, but also may deliver these drugs to other inflammation related diseases lesions. However, the treatment of tumor is far from that of inflammation. Whether it is chemotherapeutic that plays a cytotoxic role or immunotherapy agent that promote the local inflammatory response to tumor, the undesired delivery of these drugs to inflammatory lesions may bring serious injure for inflammation related diseases.

In addition to these loaded drugs may play an influence on diseases, researchers have found that biomaterials and nano-vehicles can also participate in the therapy of diseases through regulation of ROS or immunity [15–17]. As a type of terminally differentiated cells, the functional state of macrophages is plastic in response to different stimulus [18–19]. Some biomaterials and nanoparticles have been proved to regulate the function of macrophages by activating specific receptor on macrophage [16, 20], which further magnifies the risks derived from the lack of specific targeting abilities of MTDSs to a certain disease. Although some researchers intended to precisely target tumor or inflammation disease by respectively employing targeting motifs to recognize the specific antigen or receptors on M1 [21–23] or
M2 macrophages [24–26], the differentiating ability towards diseases has not been evaluated in these studies, and due to the influence of a large number of phagocytes in the peripheral blood, such strategies do not seem reliable. For example, M2 macrophages express a large number of macrophage mannose receptor and scavenger receptor higher than M1 macrophages, but a few nanoparticles have been successfully targeted deliver to tumor and atherosclerosis in different studies [27–31], through modification with dextran, a specific ligand of MMR and scavenger receptor [32].

In order to elaborate the distribution and disease influence of dextran modified nanoparticles in undesired disease, we systematically evaluate the targeting ability and effect of dextran modified polystyrene nanoparticles to different macrophages both in vitro and in vivo, through a family of surface modified polystyrene nanoparticles with similar size, charge and morphology to avoid the phagocytic effect of macrophages derived from non-focus factors.

**Result And Discussion**

**In vitro targeting ability of DEX-PS to M2 macrophages.**

Nile red-labeled polystyrene nanoparticles at size of 500 nm with similar fluorescence properties, size distribution and surface potential (Figure S1), including dextran functionalized polystyrene NPs (DEX-PS), carboxyl-functionalized polystyrene NPs (COOH-PS) and un-functionalized polystyrene NPs (PS), were employed in this study for ensuring that nanoparticles can reach the disease focus through the EPR effect [33–34] and can only be specifically phagocytosed by macrophages [35–37]. And these NPs have been proved to be not cytotoxic to Raw 264.7 cells (a murine monocytes/macrophages cells line) at particle number/cells ratio in the range of 6.25–100 (Figure S2a), while showing similar fluorescent intensity at same concentration (Figure S2b). So, PS, COOH-PS and DEX-PS at particle number/cells ratio of 100 were employed in our further experiment. In addition, M1 and M2 macrophages were respectively polarized from Raw 264.7 cells by stimulation of LPS and IFN-γ or IL-4, and verified by the determination of surface markers, cytokines and morphological characteristics by flow cytometry (FC), ELISA, PCR and phase contrast microscopy (Figure S3-6). Because of the strong phagocytosis ability of macrophages, the uptake rate of macrophages towards nanoparticles approached 100% after co-incubation for 24 h (Figure S7). Hence, the incubation time for uptake in this study was limited to less than 4 hours to observe differences in the phagocytosis ability of macrophages towards different polystyrene nanoparticles.

To verify the dextran modification on improving the targeting ability of NPs to M2 macrophages, the internalization process of various NPs by M1 or M2 macrophages was firstly monitored. After incubating with various NPs for 4 hours, the total uptake amount of DEX-PS by M2 macrophages, reflected by the mean fluorescence of whole cells, was much higher than that of other NPs (Fig. 1a). Although the uptake of PS and COOH-PS by M1 macrophages was significantly higher than that of M2 macrophages, dextran modification reversed this trend that the phagocytosis of DEX-PS by M2 macrophages was significantly higher than that by M1 macrophages (Fig. 1a). Based on the analysis of the proportion of macrophages...
that ingest nanoparticles and the average fluorescence intensity of these cells that phagocytize nanoparticles, it suggested that the M2 specific phagocytic characteristics brought by dextran modification are mainly due to the fact that dextran modification improves the phagocytic ability of a single M2 macrophage to nanoparticles (Figure 1b). On the contrary, the decrease of phagocytosis of M1 macrophages caused by dextran modification was mainly due to the decrease of the proportion of cells involved in the phagocytosis of DEX-PS (Figure 1c). These findings were further verified by the fluorescent images obtained at 4 h (Figure 1d, f). In addition, the uptake of DEX-PS by M1 macrophages was lower than that of PS and COOH-PS at all time points, while that of M2 macrophages was higher than that of PS and COOH-PS at all time points (Figure 1e, g).

In order to reveal the reasons for the improved internalized of DEX-PS by M2 macrophages, the internalization mechanism of DEX-PS and other PS NPs by differential macrophages was studied via pretreating macrophages with various uptake inhibitors prior to polystyrene nanoparticles incubation. We found that M1 macrophages took up DEX-PS mainly via cytochalasin B inhibited phagocytosis and MMR dependent uptake pathway (Figure 1h), M2 macrophages took up them via scavenger receptors dependent pathway, phagocytosis, macropinocytosis, caveolae mediated endocytosis, but mainly via MMR dependent uptake pathway (Figure 1i). Under the stimulation of dextran, the expression of MMR on M1 macrophages was greatly improved after incubation with DEX-PS for 4h (Figure S8), which explained the increase of the uptake rate of DEX-PS by M1 macrophages after incubation with them for 1 hour. In contrast, the uptake mechanism of unmodified PS and COOH-PS by M1 macrophage and M2 macrophage was both more irregular, which was a combination of multiple mechanisms (Figure S9).

To further verify the role of MMR on uptake behavior of DEX-PS by macrophage, the expression of MMR was stained and analyzed after M2 macrophages incubating with DEX-PS for 4 h(Figure 1j, S10). The proportion and average fluorescence intensity of Nile Red positive cells in MMR⁺ macrophages were both much higher than that in MMR⁻ macrophages(Figure 1j, S10). These results suggested that MMR⁺ macrophages were more likely to take up DEX-PS than MMR⁻ macrophages, and their capacity to take up DEX-PS was also higher than MMR⁻ macrophages, which further verified that the improved uptake of DEX-PS by M2 macrophages was mainly due to its specific recognition by mannose receptor [38]. Because the expression of MMR in M2 macrophage is much higher than that in M1 macrophage (Figure S3), DEX-PS was specifically phagocytosed by M2 macrophages in vitro.

**In vitrotargeting ability of DEX-PS to different macrophage subsets related diseases.**

It is of great importance to verify that the specificity in internalizing nanoparticles by M2 macrophages *in vitro* can also be conducted *in vivo*, so that they can precisely distribute in M2 related tumors, rather than in other inflammatory area mainly infiltrated with M1 macrophages. Thus, we furtherly observed the *in vivo* distribution of nanoparticles in acute peritonitis and in tumor. To avoid the interference derived from the different clearance rate of various NPs in peripheral blood, the time point of 8 h after i.v. injection of NPs was employed to observe the *in vivo* distribution of NPs. At that time, no significant difference of Nile red fluorescence intensity was found between groups (Figure S11).
As expected, obviously enhanced fluorescence signal was found in tumor of mice treated with DEX-PS both in vivo and ex vivo (Figure 2a, b), while both COOH-PS and PS were failed to target tumor lesions. In addition, DEX-PS showed high distribution in heart, liver and spleen, while PS and COOH-PS showed much weaker fluorescence there (Figure S12). Further observation showed that these DEX-PS in tumor were mainly distributed in M2 macrophages (Figure 2c). These results showed that DEX-PS successfully reached the tumor site by targeting M2 macrophages. However, we found that the active targeting ability of DEX-PS to M2 macrophages in vitro did not avoid the targeting distribution of DEX-PS to M1 macrophages related disease, acute peritonitis. DEX-PS also showed a good targeting ability to acute peritonitis (Figure 2d). In contrast, PS and COOH-PS showed no significant fluorescence at peritonitis lesions, as similar as that in tumor-bearing mice (Figure 2d). These results suggested that DEX-PS, which could be specifically recognized by M2 macrophages in vitro, lacked the ability to precisely distinguish different macrophages subtype related diseases in vivo.

**In vivo fate of DEX-PS in peripheral blood**

In order to understand the inconsistent behavior of DEX-PS in vivo and in vitro, we needed to further investigate the fate of NPs after i. v. injection. Based on this, we monitored the fluorescence intensity changes of peripheral blood derived from mice treated with various NPs in the first 12 h (Figure 3a). Despite PS treated mice showed much higher fluorescence intensity at peak than COOH-PS and DEX-PS did, COOH-PS and DEX-PS treated mice showed much more stable peak fluorescence intensity, while only tiny descent was found on them in the next 8 h, but a sharp descent at 8 h was found in PS treated group (Figure S11). These results showed that DEX-PS was cleaned up more slowly in peripheral blood than other NPs.

In blood cells, DEX-PS treated mice showed the lowest Nile Red positive cells than others (Figure 3b), however, the mean fluorescence intensity was much higher than that in PS or COOH-PS treated mice (Figure 3c). So, the total fluorescence intensity in those positive cells was much higher than other ones (Figure 3d), and these positive cells were all mainly composed by blood monocytes (CD11b+ B220-) (Figure S13). Correspondingly, the Nile Red fluorescence was also mainly displayed in blood monocytes, only little of them was distributed in B lymphocytes (CD11b+ B220+) (Figure 3d). Furthermore, there was no difference in the distribution of PS and COOH-PS in the Ly6C<sup>hi</sup> and Ly6C<sup>low</sup> monocytes, while DEX-PS was obviously phagocytized more by Ly6C<sup>hi</sup> monocytes than by Ly6C<sup>low</sup> monocytes (Figure 3d). Moreover, compared with PS and COOH-PS, more Ly6C<sup>hi</sup> monocytes were involved in the phagocytosis of DEX-PS (Figure 3e), and the average fluorescence intensity of these monocytes was much higher (Figure 3f).

It is well-known that macrophages in lesion local are usually derived and activated from Ly6C<sup>hi</sup> monocytes in peripheral blood, and some reports have shown that many kinds of NPs can be delivered to lesion local under the assistance of Ly6C<sup>hi</sup> monocytes [8]. Hence, the reason that DEX-PS were both delivered to tumor and acute peritonitis in vivo was probably due to the high uptake of them by Ly6C<sup>hi</sup> monocytes in peripheral blood, rather than the recognition of M2 macrophages in lesion.
In vitro study on the enhanced internalization of DEX-PS by monocytes.

To further understand how dextran modification affected on the ability of monocytes to uptake NPs, the NPs engulfed by undifferentiated Raw 264.7 cells (for simulating monocytes) were evaluated. After incubating with various NPs for 4 hours, the total uptake amount of DEX-PS by Raw 264.7 cells, was much higher than that of PS and COOH-PS (Figure 4a), which was mainly contributed by the improved phagocytic ability of a single Raw 264.7 cells to DEX-PS (Figure 4b), while no difference in percent of Nile Red positive cells was found between DEX-PS and other groups (Figure 4c). These findings were further verified by the fluorescence images obtained at 4 h (Figure 4d). Additionally, although the total amount of nanoparticles within cells suggested that PS were initially internalized with a high efficiency in the first 2 h, but it got gradually slowed down after then, whereas the nanoparticles within Raw 264.7 cells incubating with DEX-PS sharply boosted after 1 hour, and finally boosted to be more than that of cells with PS (Figure 4e). These results suggested that early engulfed DEX-PS by Raw 264.7 cells may in turn promote further uptake of DEX-PS by Raw 264.7 cells. In contrast, the uptake of PS and COOH-PS by a single Raw 264.7 cell tends to be saturated with time.

Then, the experiment to reveal the endocytosis mechanism of NPs by Raw 264.7 cells was further conducted. For DEX-PS, Raw 264.7 cells took up them mainly by scavenger receptors and MMR dependent pathway and cytochalasin B inhibited phagocytosis (Figure 4f). And interestingly, after incubated with DEX-PS for 4 hours, the MMR expression of Raw 264.7 cells upregulated significantly to 160 % with time (Figure 4g, h). In contrast, the internalization mechanisms of PS and COOH-PS by Raw 264.7 cells involved various pathways excluding the MMR pathway (Figure S14). Meanwhile, no obvious changes of MMR expression in Raw 264.7 cells incubated with PS or COOH-PS was observed (Figure 4h). Taken together, although the expression of MMR in Raw 264.7 cells which did not differentiate into macrophages was very low, under the stimulation of DEX-PS, the expression of MMR on Raw 264.7 cells would be up-regulated to improve the phagocytosis ability to DEX-PS by MMR pathway. These results explained the strong phagocytic ability of monocytes to DEX-PS in vivo.

In vitro effect of DEX-PS on the function of macrophages.

Both the mannose receptor-pathogen interaction and the activation of scavenger receptors pathway [39] can initiate series of signaling pathways directing to the production of lysosomal enzymes [40], ROS [41], and pro-inflammatory cytokines, such as IFN-γ, TNF-α and IL-12 [42-43]. Hence, the effect of DEX-PS, COOH-PS and PS on the function of macrophages was monitored. DEX-PS showed an obvious effect to regulate monocyte/macrophage by significantly stimulating Raw 264.7 cells, M1 and M2 macrophages release pro-inflammatory cytokines (TNF-α, IL-1β) (Figure 5a, b), but inhibiting M2 macrophages from secreting the anti-inflammatory cytokines (IL-10, TGF-β) (Figure 5c, d). In contrast, although COOH-PS could stimulate the increase of IL-1β expression in M2 macrophages (Figure 5b), they also increased the expression of TGF-β in various macrophages (Figure 5d), which did not represent a change in the anti-inflammatory or pro-inflammatory function of macrophages. In addition, after incubating with NPs for 4 h, DEX-PS obviously upregulated the CD86 expression on Raw 264.7 cells and M2 macrophages (Figure
5e, f), although DEX-PS has no significant effect on CD86 expression of M1 macrophages (Figure S15). These results suggested that macrophages tend to play pro-inflammatory functions after ingesting DEX-PS, the polarized M2 macrophages and Raw 264.7 cells were gradually transformed into M1-like macrophages with pro-inflammatory function.

**In vivo effect of DEX-PS on different macrophage subsets related disease.**

It is well known that acute peritonitis is a highly lethal macrophage related disease. The zymosan induced acute peritonitis model and acetic acid induced acute peritonitis model were both employed to evaluate the effect of DEX-PS on these inflammatory macrophage related diseases. In mice with zymosan induced acute peritonitis, the peritoneal inflammation degree was obviously enhanced by DEX-PS that TNF-α and IL-1β in peritoneal cavity was significantly upregulated (Figure 6a, b). As a result, the survival rate of DEX-PS treated mice was lower than other groups (Figure 6c), with a shortest average survival time in all groups (Figure S16), while PS or COOH-PS has not shown any influence on the survival time (Figure 6c, S16). In another acute peritonitis model induced by acetic acid, similar results were observed that DEX-PS significantly improved the expressions of TNF-α and IL-1β in extracted abdominal dropsy (Figure 6d), and hence speeded the death of mice (Figure 6e). Obviously, these results suggested that DEX-PS could significantly aggravate local inflammation by regulation of macrophages, derived from the non-specifically transporting by monocytes in vivo. Therefore, when dextran is employed for targeting therapy, such as tumor, besides focusing on its targeting efficiency, the potential pro-inflammatory effect of dextran-coated NPs in inflammation related complication should be highly concerned.

Different from acute inflammation, inflammatory macrophages play an active role in inhibiting tumor progression. Therefore, we speculated that the regulation of DEX-PS on macrophages might be beneficial to cancer therapy. First, the effect of DEX-PS treated macrophages was in vitro evaluated in Raw 264.7-4T1 co-culture system (Figure 6f). After 24 h, Raw 264.7 cells treated with DEX-PS induced a much higher proportion of apoptosis in 4T1 cells than those not treated with NPs (Figure 6g). Meanwhile, the cell viability of 4T1 cells after incubated with DEX-PS treated Raw 264.7 was accordingly lower than that of cells with untreated Raw 264.7 (Figure 6h). These results suggested that the uptake of DEX-PS might lead macrophage to be pro-inflammatory by releasing TNF-α and IL-1β, which would hence induce apoptosis of tumor cells. Furthermore, in Balb/C mice bearing 4T1 tumors, the pro-inflammatory cytokines, TNF-α and IL-1β, were increased expressed in tumor, while IL-10 and TGF-β levels were decreased, after 16 days treatment of DEX-PS (Figure 6i). Although changes of inflammatory cytokines were also observed in tumor of PS or COOH-PS treated mice, them were in swinging between pro-inflammatory and anti-inflammatory orientations (Figure 6i). In addition, the proportion of M1 macrophages in tumors treated with DEX-PS was much high than that in other groups (Figure 6j). More importantly, many DEX-PS was found in these M1 macrophages with highly expression of iNOS (Figure S17), although these DEX-PS was firstly distributed in M2 macrophages that express high degree of CD163 (Figure 3c). These results suggested that DEX-PS can reeducate tumor associated macrophages to pro-inflammatory phenotype, and thus play a regulatory role in the immune microenvironment of tumors.
In a word, we demonstrated that DEX-PS played a double sword effect in different macrophage subsets related diseases, which aggravate local inflammation in acute peritonitis, but reconstruct pro-inflammatory tumor microenvironment in tumor.

**Conclusion**

In conclusion, we show that proper surface engineering of polystyrene NPs with a specific ligand, dextran, can specifically enhance the recognition of NPs by M2 macrophages *in vitro*, via macrophage mannose receptor, rather than M1 macrophages. However, the differentiate capability of NPs towards different macrophages subtypes related diseases *in vivo* is obstructed by Ly6C<sup>hi</sup> monocytes in peripheral blood. Under the transportation by these monocytes, dextran modified polystyrene NPs are delivered not only to M2 macrophage related tumor, but also to M1 macrophages related acute peritonitis. More importantly, dextran modification would stimulate macrophages releasing more pro-inflammatory cytokines, and further exacerbated the inflammation in local of acute peritonitis, but reconstructs pro-inflammatory microenvironment in tumor that is beneficial to tumor therapy. In addition, the surface modification of NPs may lead to an unexpected regulation of macrophage function resulting positive or negative role in disease progression. Hence, it was of great necessity and importance to investigate the complex relationship between vehicles and monocytes/macrophages for designing an optimum drug delivery system for a specific macrophage related disease, which is significantly important for active targeting therapeutic or diagnostic strategies.

**Methods**

**Materials.**

Nile red labeled polystyrene NPs (PS), carboxy functionalized polystyrene NPs (COOH-PS) at a mean diameter of 0.50 µm in a 1% aqueous suspension were purchased from Spherotech, Inc. (Lake Forest, IL, USA). The fluorescence excitation and emission spectra maxima were all at 517 nm and 560 nm, respectively. All cell culture media were obtained from Gibco/Life Technologies. Dextran amine at MW of 6 k Da, was purchased from Creative PEGWorks (Chapel Hill, NC, USA). Other chemical reagents used for NP synthesis were purchased from Sigma-Aldrich (St Louis, MO, US).

**Synthesis of dextran modified polystyrene NPs.**

COOH-PS at number of 2.5 \times 10^6 and 5 nmol of dextran amine were co-dispersed in 100 µL of 0.1 M 2-N-morpholino ethane sulfonic acid (MES) solution. Then 0.3 mg of EDC (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) were twice added in, with a vortexing and incubating process of 20 minutes at ambient temperature for each time. After incubating for another 80 minutes on a rotary mixer, the product was centrifuge and remove the supernatant carefully. Then the obtained pellet was suspended in 1 mL of 0.1 M PBS containing 0.02% Tween-20.

**Kinetics of internalization of various NPs by macrophages.**
Phagocytosis assays of different phenotype macrophage were performed by incubating different surface functioned NPs with Raw 264.7 cells, M1 macrophages or M2 macrophage. The polarization from Raw 264.7 cells to M1 or M2 macrophages were verified by the determination of surface markers, cytokines and morphological characteristics by flow cytometry (FC), ELISA, PCR and phase contrast microscopy. As the fluorescence properties of various NPs, including fluorescence intensity at the same concentration, have no significant difference, the concentration of NPs employed here was fixed on the particle number/cells ratio of 100. Because the uptake rate of macrophages towards NPs approached 100% after co-incubation for 24 h, the incubation time for uptake was limited to less than 4 h. After incubating at 37 °C for 0.5, 1, 2 and 4 h, quantification of phagocytosis by FC was performed and analyzed after the macrophages were washed and detached by the FACS Aria II flow cytometer (BD) for median fluorescence intensity of the cell population in the PE channel. In addition, macrophages incubated with various NPs for 4 h were collected and observed by Zeiss LSM 710 laser-scanning microscope (Carl Zeiss, Germany).

Biodistribution of Various NPs in tumor-bearing mice.

Tumors were established by inoculating 4T1 cells subcutaneously in Balb/C mice. When the tumors reached to 100 mm$^3$, 100 µL 1% various PS were i. v. administrated. After 8 h, the biodistribution of Nile Red in mice were imaged using a living imaging system (IVIS Spectrum, PerkinElmer). In order to avoid the influence of strong spontaneous fluorescence of the surrounding hairs, the tumor region was delineated as ROI separately, and this ROI region was employed to analyze the fluorescence distribution in tumor using the Living image software 4.3. After imaging, the mice were sacrificed to harvest the main organs and tumor for ex vivo imaging using this living imaging system.

Biodistribution of Various NPs in mice with zymosan induced acute peritonitis.

Male Balb/C mice were i.p. injected with 1 mL of zymosan suspension in saline (1 mg/mL) to induce acute peritonitis, then randomly allocated into 4 groups. After 16 h, mice in various groups were respectively i. v. injected with 100 µL 1% various PSs or saline as control. After 8 h of the injection, the biodistribution of Nile Red in mice were imaged using a living imaging system (IVIS Spectrum, PerkinElmer). In order to avoid the influence of strong spontaneous fluorescence of the surrounding hairs, the abdominal region was delineated as ROI separately, and this ROI region was employed to analyze the fluorescence distribution in tumor using the Living image software 4.3.

Treatment of Peritonitis.

For zymosan induced acute peritonitis, male Balb/C mice were i.p. injected with 1 mL of zymosan suspension in saline (1 mg/mL) to induce acute peritonitis, then randomly allocated into 4 groups. After 12 h, mice in various groups were respectively i. v. injected with 100 µL 1% various PS or saline as control. After the injection, the survival status and survival rate of mice were recorded. To quantify the inflammatory cytokines in abdominal dropsy, 100 µL abdominal dropsy was exacted and collected after mice treated with NPs for 4 h (injected with zymosan for 4 h) via a sterile syringe. After centrifugation of
dropsy at 10621 g for 15 min at 4 °C, the concentration of TNF-α, IL-1β, IL-10 and TGF-β in the
supernatant were measured by ELISA under the guide of kit instructions. Similar treatment was
conducted for male Balb/C mice with acetic acid induced acute peritonitis, which was induced by i.p.
injection of 0.1 mL acetic acid solution in saline (3%).

Treatment of tumor.

Tumors were established by inoculating 4T1 cells subcutaneously in Balb/C mice. After 2 weeks, when
the tumors reached to 100 mm³, 100 µL 1% various PS were i. v. administrated every 4 days. After 16
days, the mice were sacrificed, and the tumor tissues were isolated and divided into two parts. To
quantify the inflammatory cytokines in tumor, 100 µg tumor tissue was homogenate for evaluating of the
levels of inflammatory cytokines. In addition, immunofluorescence analysis was also conducted to
examine expression of iNOS and CD163 in the tumor, using a Zeiss LSM confocal system.

**Statistical analysis.**

All data are expressed as mean ± standard deviation (SD) and analyzed by the software of SPSS 18.0
statistical package. For experiment with two groups, an unpaired t-test was performed in statistical
analyses of independent continuous variables, while one-way ANOVA test with two-tailed Student’s t-test
was employed in experiments with three or more than three groups. Statistical significance was assessed
at p < 0.05.

**Declarations**

- **Ethics approval and consent to participate:** The study had animal ethics approval from The Army
Medical University Animal Ethics Committee. The manuscript does not contain clinical studies or
patient data.
- **Consent for publication:** Not applicable.
- **Availability of data and materials:** The datasets used and analysed during the current study are
available from the corresponding author on reasonable request.
- **Competing interests:** The authors declared that they have no conflicts of interest.
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- **Author’s contributions:** YY and LL participated in experiments, data analysis and manuscript writing
and contributed equally to this work. YL assisted with preparation of nanoparticles. CP supported
biological study. YT and XZ helped with data analysis. SL provided technical supports. CZ, XZ and
XL supervised entire project and involved in the designing of conceptual framework and revised the
manuscript. All authors read and approved the final manuscript.
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Figures
Figure 1

The uptake behavior of DEX-PS by different macrophage subsets. (a-c) The mean fluorescence of whole cells (a), mean fluorescence of Nile Red positive cells (b) and percent of Nile red+ cells (c), after incubating with various NPs. (d, e) The CLSM images of M1 macrophages (d) and M2 macrophages (e)
that incubated with PS, COOH-PS and DEX-PS for 4 h. (f, g) Kinect profile of mean fluorescence of whole M1 macrophages (f) and M2 macrophages (g). (h-i) The uptake rate of DEX-PS by M1 (h) and M2 macrophages (i) influenced by various inhibitors. (j) The fluorescence distribution of DEX-PS in MMR+ and MMR- cells. Scale bars in (d) and (e) indicated 20 μm.* means p<0.05
In vivo targeting ability of DEX-PS to tumor and acute peritonitis. (a) Typical in vivo images illustrating Nile Red fluorescent signals in tumor bearing mice. (b) Ex vivo images illustrating Nile Red fluorescent signals in isolated tumors. (c) The distribution of NPs in tumor section observed by CLSM. (d) In vivo distribution of Nile Red fluorescent signals in acute peritonitis. The area marked by black dotted line is ROI (region of interest) and the area marked by yellow solid line is the focus of disease.
Figure 3

In vivo fate of DEX-PS in peripheral blood. (a) Ex vivo images illustrating Nile Red fluorescent signals in isolated whole blood. (b) The percent of Nile Red positive cells in blood cells. (c) The mean fluorescence intensity of Nile Red in Nile Red positive cells. (d) The total fluorescence intensity of Nile Red in Nile Red positive cells. (e) The percent of CD11b+ Ly6Chi cells in Nile Red positive monocytes cells (CD11b+B220-). (f) The mean fluorescence intensity of CD11b+ Ly6Chi cells in Nile Red positive monocytes cells. * means p<0.05.
Figure 4

The improved uptake of DEX-PS by Raw 264.7 cells. (a-c) The mean fluorescence of whole cells (a), mean fluorescence of Nile Red positive cells (b) and percent of Nile red+ cells (c) after incubating with various NPs; (d) The CLSM images of Raw 264.7 cells after incubating with PS, COOH-PS and DEX-PS for 4 h. (e) Kinect profile of mean fluorescence of whole Raw 264.7 cells. (f) The uptake rate of DEX-PS by Raw 264.7 cells influenced by various inhibitors. (g) Dynamic changes of mean intensity of MMR on Raw 264.7 cell after incubation with DEX-PS. (h) Relative expression changes of MMR on Raw 264.7 cell after 4 h incubation with PS, COOH-PS and DEX-PS, respectively. Scale bars in (d) indicated 20 μm.* means p<0.05.
Figure 5

The regulation of various PS on the function of macrophages. (a-d) The typical cytokines expression in Raw 264.7 cells, M1 and M2 macrophages. (e, f) The expression change of CD86 on Raw 264.7 cells (e) and M2 macrophage (f) after treated by various NPs. * means p<0.05, vs. untreated.
Figure 6

The effect of DEX-PS on the progression of macrophage related diseases. The typical cytokines expression (a, b) in abdominal dropsy and the survival rate (c) of mice administered with various NPs after peritonitis induced by i.p. injection of zymosan; The typical cytokines expression (d) in abdominal dropsy and the survival rate (e) of mice administered with various NPs after peritonitis induced by i.p. injection of acetic acid; (f) Scheme of macrophage-tumor cell co-culture system; (g) Apoptosis proportion
of 4T1 tumor cells in co-culture system determined by tunnel assay; (h) Cell viability of 4T1 tumor cells in various co-culture systems; (i) The typical cytokines expression in tumors isolated from various NPs treated 4T1 bearing mice; (j) The typical immunofluorescence images of tumors isolated from various NPs treated 4T1 bearing mice. * In (a), (c) and (i) means p<0.05, vs. saline. * in (g) and (h) means p<0.05. Scale bar indicated 100 μm

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