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A simple and unique strategy has been successfully designed for sensitive detection and rapid clearance of bacterial lipopolysaccharides (LPS) by integration of core–shell Fe₃O₄@SiO₂ magnetic nanoparticles with a perylene–diimide (PDI) conjugated LPS-recognition peptide.

Bacterial lipopolysaccharides (LPS), key components in the outer membrane of Gram-negative bacteria, are well known as bacterial endotoxins that may cause serious septic shock or many other health problems in the intensive care.¹ The extreme toxicity of bacterial LPS has thus urgently initiated extensive research efforts toward the development of novel and specific strategies to sensitively identify, and more importantly, to rapidly remove such highly toxic endotoxins from biological and pharmaceutical products. One commonly used FDA-approved technique for LPS detection is the enzymatic limulus amoebocyte lysate (LAL) assay. However, this assay requires a rather long and complicated testing procedure, and moreover, the results from the LAL assay are also susceptible to environmental variations, especially in pH and temperature.² Very recently, several fluorescent, colorimetric or electrochemical sensors based on small molecules like,³ CD14-derivatized peptides,⁴ liposomes,⁵ or polymers⁶ have been reported for sensitive determination of bacterial LPS in aqueous solutions. Although all of these attempts have shown great promise with their individual detection limit range (from micromolar to picomolar level),^{5a,6} none of these assays indicated the capacity for the effective separation of LPS from the detection mixtures. Given the clinical importance of LPS, a simple and specific sensing strategy with more promising functions to clear bacterial endotoxins remains a big challenge in current research. Although several conventional

techniques such as ultrafiltration, two-phase extraction, chromatography and functionalized nanoparticles have been utilized to remove LPS contaminants from certain biological preparations,⁷ these techniques may not report the specific and sensitive determination of LPS directly, and their LPS clearance efficiency could be potentially affected by environmental changes (e.g. pH).^{7b,c} Therefore, the development of a stable and easily controlled method with a combination of sensitive detection and efficient clearance of LPS in complex biological samples such as bacterial cell lysates and human serums will be of great importance in the pharmaceutical industry, and such highly desirable studies have not been fully explored yet.

Here we first present simple and novel dual-functional magnetic nanoparticles (MNPs) for effective detection and removal of bacterial LPS by introducing a specific perylene diimide (PDI) conjugated LPS-binding peptide onto the surface of nanoparticles. PDI derivatives were chosen mainly because of their high thermal and photostability, high quantum yield, and more importantly, their favorable self-assembly properties in aqueous solutions.⁸ Along with our continuous efforts in selective bacterial imaging and photo-inactivation of pathogens,⁹ a set of designed LPS recognition peptides were screened that exhibited promising binding affinity for the bacterial surfaces. In this rational design, a simple and high-affinity LPS binding peptide with the YVLWKRKRKFCFI-NH₂ sequence was selected as the targeting ligand to conjugate with magnetic Fe₃O₄@SiO₂ core–shell structures through a short linker containing PDI as a fluorescent reporting group. In the absence of LPS, the fluorescence of the PDI molecules may undergo self-quenching due to the strong $\pi \cdots \pi$ stacking interactions.¹⁰ However, in the presence of LPS, the specific LPS binding would trigger conformational changes in the peptide⁹ that may lead to the disruption of the hydrophobic stacking between adjacent PDI molecules and induce a significant fluorescence recovery (Fig. 1). Furthermore, the LPS captured by the recognition peptide on MNPs can be easily removed by using a simple magnet, therefore separating the toxic bacterial LPS from the contaminated biological mixture, thus lowering the risk of LPS toxicity to both the environment and human healthcare units.

In this study, the affinity ligand engineered MNPs were chosen as solid platforms because they have demonstrated great promise in

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Fig. 1 LPS recognition peptide functionalized $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core-shell MNPs for fluorescence turn on detection and clearance of LPS.

biomedical applications including cancer imaging and therapy,¹¹ bio-separation,¹² targeted drug or gene delivery,¹³ and the sorting of living cells and bio-molecules.¹⁴ The $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core-shell MNPs were prepared according to the method reported previously.^{11,15} To achieve effective particle functionalization, an azido-dPEG₄ linker was first coupled to the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ surface followed by conjugation with the alkyne modified PDI peptide moieties through "Click-chemistry" (Fig. 1). The formation of the PDI-peptide functionalized MNP ($\text{MNP}_{\text{PDI-peptide}}$) with the size distribution of about 25 nm was finally confirmed by spectroscopic and TEM analysis. The optimal amount of the peptide conjugate on $\text{MNP}_{\text{PDI-peptide}}$ was determined to be 10 nmol mg^{-1} (Fig. S1–S4, ESI†).

We further examined the possibility of $\text{MNP}_{\text{PDI-peptide}}$ responding to bacterial LPS and the induced fluorescence change in buffer solutions. Typically, 100 $\mu\text{g mL}^{-1}$ of $\text{MNP}_{\text{PDI-peptide}}$ were incubated with 100 $\mu\text{g mL}^{-1}$ of LPS in Tris-buffered saline solution (TBS, 50 mM, pH 7.4). As expected, very weak fluorescence was detected before addition of LPS in $\text{MNP}_{\text{PDI-peptide}}$ solutions (Fig. 2a), mainly due to the self-quenching caused by the $\pi\cdots\pi$ stacking of PDI on particle surfaces.¹⁰ However, upon the addition of LPS for 15 min, there was over 7-fold fluorescence enhancement at the emission wavelength of 539 nm, and no obvious further change was observed as the incubation time was increased (Fig. 2a, Fig. S5, ESI†). As a control, the MNP_{PDI} conjugate without the LPS recognition peptide was also applied to respond to LPS (Fig. 2a). No obvious fluorescence change was observed, clearly confirming that the LPS binding peptide functioned as an indispensable moiety to specifically target bacterial LPS, hence changing the conformation of stacked PDI molecules and eventually restoring the fluorescence from $\text{MNP}_{\text{PDI-peptide}}$. In addition, the analysis also showed that the fluorescence enhancement was LPS concentration dependent. The plot of fluorescence intensity at 539 nm *versus* the LPS concentration displayed a good linear relationship ($R^2 = 0.996$) with a LPS concentration range up to 20 $\mu\text{g mL}^{-1}$ (Fig. 2b), and the detection limit of LPS in TBS buffer was determined to be 280 ng mL^{-1} (Fig. S6, ESI†), which was comparable to most of the LPS sensing

Fig. 2 (a) Fluorescence spectra ($\lambda_{\text{ex}} = 450$ nm) of $\text{MNP}_{\text{PDI-peptide}}$ and MNP_{PDI} with or without LPS (100 $\mu\text{g mL}^{-1}$) in TBS buffer; (b) fluorescence of $\text{MNP}_{\text{PDI-peptide}}$ (100 $\mu\text{g mL}^{-1}$) after incubation with LPS at different concentrations (from 0, 1 to 250 $\mu\text{g mL}^{-1}$) in TBS buffer at 37 °C. The inset shows the plot and linear fitting of normalized fluorescence at 539 nm *vs.* the concentration of LPS (2–20 $\mu\text{g mL}^{-1}$); (c) the fluorescence of 100 $\mu\text{g mL}^{-1}$ $\text{MNP}_{\text{PDI-peptide}}$ in the absence or presence of LPS (100 $\mu\text{g mL}^{-1}$) under different pH conditions; (d) the selective fluorescence increase upon addition of LPS against various biologically important species (all at 10 μM).

systems reported previously.^{3a,4,5} Furthermore, the fluorescence intensity of $\text{MNP}_{\text{PDI-peptide}}$ itself at 539 nm did not change dramatically in a wide pH range from 4 to 11. Upon addition of LPS, the fluorescence intensity was increased about 4–7 times under all tested pH conditions (Fig. 2c), clearly suggesting that the as-prepared $\text{MNP}_{\text{PDI-peptide}}$ could serve as an environmentally stable and reliable platform for sensitive LPS detection.

Furthermore, a selectivity study was also performed by incubating $\text{MNP}_{\text{PDI-peptide}}$ with various small or macro-biomolecules (Fig. 2d, Fig. S7 and S8, ESI†). Compared to the results obtained from $\text{MNP}_{\text{PDI-peptide}}$ with LPS, no significant fluorescence response was detected against all the tested analytes at concentrations up to 10 μM including the highly negatively charged BSA or DNA, which has been reported to potentially interfere with LPS detection,^{5a} confirming that the specific LPS-peptide interaction could easily replace the non-specific electrostatic attraction between the negatively charged bio-molecules and LPS affinity peptides. The conformational change in the activated peptide⁹ will greatly induce the recovery of the quenched fluorescence by disrupting $\pi\cdots\pi$ stacking of PDI on the particle surface. These results indicated that the $\text{MNP}_{\text{PDI-peptide}}$ could work as a highly selective fluorescent turn-on sensor for toxic LPS determination.

More importantly, we also exploited the feasibility of using $\text{MNP}_{\text{PDI-peptide}}$ to remove the toxic bacterial components. Typically, the procedure to remove LPS consisted of two simple steps: (1) mixing of $\text{MNP}_{\text{PDI-peptide}}$ with suspension of lysed bacterial cells (e.g. *E. coli*, DH5 α etc.) or LPS contaminated human serum samples for 15 minutes and (2) applying a small magnet to attract $\text{MNP}_{\text{PDI-peptide}}$ to the wall of the vials to capture LPS (Fig. 3a). The optimum concentration of LPS and protein components from mixtures were determined by the LAL assay or the standard Bradford

Fig. 3 (a) Scheme of LPS removal based on $\text{MNP}_{\text{PDI-peptide}}$, (b) quantified magnetic LPS removal and protein recovery from *E. coli* cell lysates, (c) NO generated from RAW 264.7 cells pre-treated with serum before and after LPS detoxification. Blank: serum treated cells without LPS.

method, which were used to evaluate the LPS clearance efficiency.^{1,2} As shown in Fig. 3b, the removal of LPS was strongly dependent on the concentration of $\text{MNP}_{\text{PDI-peptide}}$ and effective LPS removal (e.g. over 87% from lysate of 10^9 cfu mL^{-1} *E. coli* cells) could be easily achieved when $400 \mu\text{g mL}^{-1}$ of $\text{MNP}_{\text{PDI-peptide}}$ was used, while no obvious loss of the total proteins was observed during the process of LPS removal. Moreover, after a simple multi-cycle removal (e.g. 3 or 4 cycles) with a fixed amount of $\text{MNP}_{\text{PDI-peptide}}$ (e.g. $200 \mu\text{g mL}^{-1}$) each time, the LPS could be completely cleared from about 6800 endotoxin units (EU) per mL and there was even less than 1 EU mL^{-1} left in the bacterial lysates (Fig. S9a and S10, ESI[†]). In contrast, when similar clearance was conducted with Gram-positive *B. subtilis* cell lysates, only non-specific background signals were observed before and after treatment, suggesting that $\text{MNP}_{\text{PDI-peptide}}$ could selectively remove the LPS components from Gram-negative bacteria. Moreover, we also examined the feasibility of $\text{MNP}_{\text{PDI-peptide}}$ to detoxify LPS contamination by efficient removal of LPS endotoxins from human serum samples. Similarly, LPS removal strongly depends on the concentrations of $\text{MNP}_{\text{PDI-peptide}}$ and more efficient LPS clearance (e.g. >89%) could be easily achieved at higher concentrations of $\text{MNP}_{\text{PDI-peptide}}$ (e.g. 2.0 mg mL^{-1}), even only upon a single cycle particle separation (Fig. S9b, ESI[†]). Meanwhile, the detoxification effect after removal of LPS was also evaluated in macrophage cultures by measuring the concentration of nitrite,¹⁶ a stable metabolite of NO that has been well established as a pre-inflammatory stimulus to reflect the cell stress upon stimulation by bacterial LPS (Fig. 3c). Compared to the control study without any LPS in the serum, the macrophage RAW 264.7 cells incubated with the LPS contaminated serum for 24 hours would obviously induce the generation of a high level of nitrite, indicating the occurrence of cell stress stimulated by LPS. In contrast, incubation of serum under similar conditions but after efficient LPS removal resulted in a low level of nitrite generation. The decreasing nitrite concentration observed in a macrophage culture medium was consistent with the lower level of remaining LPS after effective separation using MNPs (Fig. 3). Importantly, the $\text{MNP}_{\text{PDI-peptide}}$ itself did not demonstrate obvious cytotoxicity (e.g. cell viability of 87%) even at the concentration of nanoparticle conjugate up to $400 \mu\text{g mL}^{-1}$ (Fig. S11, ESI[†]), suggesting that a trace amount of the residue

$\text{MNP}_{\text{PDI-peptide}}$ in the whole cell environment during the magnetic LPS removal would not be sufficient to induce obvious cellular toxicity.

In summary, by combining $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core-shell MNPs with a specific affinity ligand based on a PDI conjugated LPS recognition peptide, we first developed a simple and novel strategy for effective fluorescence detection of LPS and rapid clearance of such toxic bacterial endotoxins. Based on the specific interactions between LPS and affinity peptides on the surface of MNPs, self-stacking of adjacent PDI molecules was disrupted, which broke self-fluorescence quenching and thus restored the fluorescence. This peptide functionalized MNPs can sensitively identify LPS with the promising detection limit down to the nano-molar level and it is highly selective for bacterial LPS when compared to other interfering bio-analytes. More importantly, such simple and effective LPS detection nanoparticles also indicated the low risk of LPS toxicity to the environment and human healthcare by rapidly clearing bacterial LPS from complicated bacterial lysates and LPS contaminated serum samples. We envision that such simple and novel development would hold great potentials for the future treatment of bacteria associated sepsis with minimum complicated sample pre-treatment in extensive clinical practice.

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