Supplementary Materials for

A nanoparticulate dual scavenger for targeted therapy of inflammatory bowel disease

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MATERIALS AND METHODS

Materials and Reagents
Branched polyethyleneimine (PEI, Mw 25 kDa), calf thymus DNA (ct-DNA), tetraethyl orthosilicate, cetyltrimethylammonium tosylate (CTAT), triethanolamine, γ-chloropropyl trimethoxysilane, 3-aminopropyltriethoxysilane, fluorescein isothiocyanate (FITC), and reduced glutathione (GSH) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Selenium powder, sodium powder, sodium borohydride, ammonium nitrate, anhydrous sodium sulfate, hydrogen peroxide (30%), and anhydrous ethanol were purchased from Beijing Chemical Reagent Co. (Beijing China). Cyanine 7 succinimidyl ester (Cy7-NHS) was purchased from Lumiprobe Corporation (Hallandale Beach, FL, U.S.A.). Dulbecco's modified Eagle's medium, fetal bovine serum, and 0.25% trypsin-EDTA were purchased from Gibco Co., Ltd. (Carlsbad, CA, USA). Antibiotic/antimycotic solution was purchased form Life Technologies, Inc. (Grand Island, NY, USA). All other reagents were commercially available and used as received.

DSS (#0216011080) was purchased from MP Biomedicals. TNBS were purchased from Sigma-Aldrich. BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit was purchased from BD Biosciences. TruStain FcX (anti-mouse CD16/32) antibody (clone 93) was purchased from Biolegend. F4/80 monoclonal antibody (cat. #11-4801-82), rat IgG2a kappa isotype control (cat. #11-4321-81), CD11c monoclonal antibody, (cat. #12-0114-82), Armenian hamster IgG isotype control (cat. #12-4888-81), and CD289 (TLR9) monoclonal antibody (cat. #11-9093-82) were purchased from eBioscience. GAPDH mouse monoclonal antibody (cat. #60004-1-Ig) was purchased from Proteintech. Anti-NF-kB p65 (phospho S536) antibody (cat. #ab28856), anti-NF-kB p65 antibody (cat. #ab16502), anti-MyD88 antibody (cat. #ab135693), anti-TLR9 antibody (cat. #ab134368), anti-FOXP3 antibody (ab215206), and anti-TGF-β antibody (ab215715) were purchased from Abcam. BCA protein assay kit was purchased from Thermo Fisher Scientific. TNF-α, IL-6, and MCP-1 were purchased from Proteintech. MDA assay kit, Reactive Oxygen Species Assay Kit, SOD assay kit, and GPx assay kit were purchased from Nanjing Jiancheng Bioengineering Institute, China. Trizol reagent was purchased from Invitrogen. SYBR green qPCR assay was purchased from Roche. cDNA synthesis kit was
purchased from Transgen Biotech, China. All primers were synthesized by Sangon Biotech, China.

GAPDH forward sense: TGTGTCGCTGGATCTGA;
GAPDH reverse sense: CCTGCTTCACCACCTTCTTGAT;
TNF-α forward sense: CACGTCGTAGCAAACCACC;
TNF-α reverse sense: TGAGATCCATGCGTTGGA;
iNOS forward sense: GAATCTTGGAGCGAGTTGG;
iNOS reverse sense: AGGAAGTAGGTGAGGGCTTG;
Arg-1 forward sense: CCGCAGCATTAAGGAAAGC;
Arg-1 reverse sense: CCCGTGGTCTCTCCTACATTG.

CpG 1826: (5’-TCCATGACGTTCCTGACGTT-3’)
CpG 2006: (5’-TCGTCGTTTTGTCGTTTTGTCGTT-3’)

**Synthesis of diselenide-bridged MON**

Briefly, 1.2 g of cetyltrimethylammonium tosylate (CTAT) and 0.3 g of triethanolamine were dissolved in 80 mL of deionized water and stirred at 80 °C for 1 h. A solution containing 4.0 g of bis[3-(triethoxysilyl)propyl]diselenide (BTESePD) and 6.0 g of tetraethyl orthosilicate was then added dropwise to the surfactant solution. The resulting mixture was stirred at 80 °C for 4 h at 1000 rpm. The products were obtained after centrifugation and repeatedly washing the reaction mixture with ethanol and refluxed in an ethanol solution of ammonium nitrate (1% w/v) for 12 h. The diselenide-bridged MON were collected, washed, and refluxed in ethanol to remove CTAT.

**Synthesis of dye-labeled MON-PEI**

Dye-labeled scavengers were prepared based on our protocol reported previously (15). Cy7-NHS were used to prepare dye-labeled MON, PEI, and MON-PEI. Briefly, 1 mg of Cy7-NHS was mixed with 10 mg of amino-functionalized MON, PEI, or MON-PEI. The mixture was shaken overnight at 4 °C, followed by removal of unreacted dye molecules via centrifugation to obtain Cy7-labeled scavengers.

**Characterization of MON and MON-PEI**
The morphology of MON was observed with a JEM-2100F transmission electron microscope (JEOL, Ltd., Japan) and a scanning electron microscope (FEI Quanta 200F). The hydrodynamic diameter and zeta potential of the nanoparticles were characterized using a Nano-ZS 90 Nanosizer (Malvern Instruments Ltd., Worcestershire, UK). The pore size distribution and specific surface area were calculated and evaluated using Barrett-Joyner-Halenda (BJH) and Brunauer-Emmett-Teller (BET) methods. The PEI content of MON-PEI was determined by thermogravimetric analysis. Degradation of MON (100 μg/mL) was evaluated in 100 μM of H₂O₂ solution at 37 °C under constant rotation. At 0, 1, and 3 days, samples were collected for TEM. Degradation of MON or MON-PEI (100 μg/mL) was evaluated in simulated gastric fluid (SGF) solution at 37 °C under constant rotation. At 12 h, samples were collected for TEM, the hydrodynamic diameter and zeta potential were determined at the same time.

**SRB assay**

After the treatment of each group, 100 μL of 30% trichloroacetic acid (TCA) was added to each well and refrigerated for 1 h at 4 °C, then the supernatant was discarded and the plate was washed 5 times with water and air dried. 100 μL of SRB solution 0.4% (w/v) in 1% acetic acid was added and incubated at room temperature for 30 minutes. Then, the plate was washed 5 times with water and air dried for discarding unbound SRB. Afterwards, 150 μL of Tris-base (pH=10.5) were added to each well for solubilizing bound SRB and the plate was shaken for 10 minutes. The absorbance was recorded at 570 nm using a Multi-Mode Microplate Reader.

**Determination of RSS-scavenging effects**

To determine the RSS-scavenging effects of MON or MON-PEI, the sample in 0.5 mL of water or 0.2 M hydrochloric acid were added 0.4 mL of 1 M ammonium hydroxide, 3.6 ml of water, and 0.5 mL of 0.5 M potassium cyanide. After incubating for 45 min at room temperature, 0.1 mL of 38% formaldehyde and 1 mL of Goldstein's reagent are added. The absorbance was determined at 460 nm.

To determine the protective effect of nanoscavengers from RSS damage, RAW264.7 were grown in 96-well culture plates at 10⁴ cells/well and were cultured. After 12 h, Na₂S₄ (1.5 mM) with or without scavengers were added to each well, and plates were incubated for 24 h. The viability of cells was measured with SRB assay described as above.

**Measurement of GPx Activity**
The enzymatic activities of glutathione peroxidase (GPx) including GPx1, 2, 3, and 4 were measured in colon tissue samples. After colon tissues were washed with saline solution, a sample from the distant colon (2 cm in length) was taken and homogenized in phosphate buffer using a Bullet Blender (Next Advance, NY, USA). Homogenates were centrifuged (10,000 × g, 15 min, 4°C), and the supernatants were collected for the tests. The enzymatic activities of GPx were determined according to the manuscript provided by the assay kits (Beyotime, China).

**Immunohistochemical staining**

Paraffin-embedded colon tissue sections were deparaffinized in xylene and rehydrated in alcohol. Antigen retrieval was performed in pH 6.0 sterile sodium citrate buffer. The slides were incubated in 3% H$_2$O$_2$ for 10 min at room temperature to block endogenous peroxidase activity and rinsed 3 times × 3 min with PBS. Following this, the slides were incubated with antibodies against TLR9 (ab37154,1:200; Abcam, Cambridge, UK), iNOS (ab115819,1:200; Abcam, Cambridge, UK), TNF (ab9739,1:200; Abcam, Cambridge, UK) or F4/80 (ab100790,1:200; Abcam, Cambridge, UK) for 1 h at 37°C and rinsed 3 times × 3 min with PBS. After that, the slides were incubated with enzyme-labeled goat anti-rat IgG for 20 min at room temperature. Then slides were rinsed twice 3 times × 3 min with PBS and immersed in diaminobenzidine (DAB) detection solution for 5 min. Sections were counterstained with haematoxylin for 1 min, dehydrated and mounted.

To precisely represent the expression level of TLR 9, TNF-α, iNOS, or F4/80, we used the H-score system in this study and evaluated the stained slides as no staining: 0, weak: 1+, moderate: 2+, and strong staining: 3+. The cells remaining positively stained were estimated as percent (0%–100%). An H-score was generated in 0–300 range by multiplying the percentage of positively stained cells and staining intensity score (H-score= 3 × the corresponding positive percentage + 2 × the corresponding positive percentage + 1 × the corresponding positive percentage + 0 × the corresponding positive percentage).

**Animals**

Animal protocols were approved by the Ethics Committee for the Use of Experimental Animals of Xi’an Jiaotong Univeristy and the Ethics Committee for the Use of Experimental Animals of South China University of Technology. Male C57BL/6 mice (Six to eight-week-old) were obtained from Beijing Vital River Laboratory Animal Technology. Mice were housed under a
12-h light / dark cycle and constant temperature (21 ± 1°C) with free access to autoclaved chow and water.

**Peritoneal macrophage isolation**

Peritoneal macrophages were isolated as previously described (15). In brief, peritoneal lavages were performed using 4 mL of PBS. Peritoneal cells were suspended in RPMI 1640 containing 10% fetal calf serum (FCS) and incubated at 37 °C in a humidified atmosphere with 5% CO₂ for 24 h. The nonadherent cells were discarded, and the adherent cells were kept for the following experiments.

**Flow cytometric analysis**

To determine the number of TLR9⁺ immune cells and percentage of M1 polarized macrophages in peritoneal fluid, the peritoneal fluid cells were collected. The cells were washed twice and resuspended in FACs buffer, and then calculated. For the number of TLR9⁺ immune cells, the cells were incubated with CD289 (TLR9) monoclonal antibody at 4 °C for 30 min. For the percentage of M1 polarized macrophages, the cells were firstly incubated with FcBlock (TruStain FeX™ anti-mouse CD16/32) for 10 min on ice to avoid nonspecific binding, and stained with F4/80 monoclonal antibody, CD11c monoclonal antibody, along with isotype controls (A rat IgG2a kappa and Armenian hamster IgG) for 30 min at 4 °C. After that the cells were washed, centrifuged at 400g for 5 min at 4 °C, and resuspended in FACs buffer. Cells were analyzed via flow cytometer (BD Accuri® C6) and BD Accuri C6 plus analysis software.

**Quantitative real-time PCR assay**

Total RNA was extracted from peritoneal macrophages, using trizol reagent, and reverse transcribed into cDNA with TransScriptII Reverse Transcriptase (TransGen Biotech, Beijing, China). qPCR (FastStart Universal SYBR Green Master mix, Roche Diagnostics Ltd, Lewes, UK) was performed using LightCycler 96 Real-Time PCR Detection System (Roche). Amplified transcripts were quantified using the comparative Ct method. The primer sequences used are shown in Materials and Reagents.

**Western blotting**

Protein expression of TLR9, MyD88, NF-kB p65 (phospho S536), NF-kB p65 and GAPDH were analyzed by Western blot analysis. Peritoneal macrophages were lysed with RIPA lysis buffer along with protease inhibitors and centrifuged. Nonreducing SDS buffer was added into the supernatant. Samples were water-heated at 100 °C for 5 min and centrifuged at 11,000 g for 5
min and stored at -80 °C. 10 μg samples were separated on 10% SDS-PAGE and transferred onto a PVDF membrane. Then, the blots were blocked with BSA and incubated with the above antibodies, followed by incubation with HRP-conjugated secondary antibody. Bands were analyzed by ECL detection system (GelView 6000Pro, BLT). Quantitative analysis was performed using Quantity One. GAPDH was used as the internal control.

Selenium level detection
Use atomic fluorescence to determine selenium level in serum. 1 mL mixture of nitric acid and perchloric acid in the ratio of 4:1 was added to 0.2 mL of serum. It was heated to emit white smoke of perchloric acid, followed by removing to cool. Then 0.5 mL of hydrochloric acid solution and deionized water were added. Fluorescence value was measure by atomic fluorescence spectrophotometer. Selenium level were calculated by using the standard curve.

Biosafety evaluation of scavengers
Normal mice were treated with 10mg/kg MON, PEI, or MON-PEI via oral gavage, IP injection, or rectal enema for 14 consecutive days, while the control mice were administered with water of the same volume. Changes of Body weight were monitored until 28 days after treatment. Mice were sacrificed on day 42. The blood samples were collected and ALT, AST, BUN, CRE levels were measured. The spleen, heart, lungs, liver, kidney, and colon of mice were collected, and stained with hematoxylin and eosin.

Normal mice were treated with MON-PEI (40 mg/kg) daily, every two days or every three days via oral gavage for 14 days. Changes of body weight were monitored until 28 days after treatment. Mice were sacrificed on day 42. The blood samples were collected for measuring ALT, AST, BUN, CRE levels. The spleen, heart, lungs, liver, kidney, and colon of mice were collected and stained with hematoxylin and eosin.

Study of frequency-dependent and dose-dependent therapeutic effects of MON-PEI via oral gavage
In the dose-dependent experiment, the 3% DSS-challenged mice were administrated with different dose frequency of MON-PEI (2.5, 5, 10, 20, 40 mg/kg) via oral gavage starting on the 3rd day and up to day 14. Mice in the control group received the same volume of PBS. Changes of body weight, DAI value, colon length, and colonic damage scores were monitored on day 14. In the frequency-dependent experiment, the 3% DSS-challenged mice were administrated with MON-PEI (10, 20, and 40 mg/kg) by gavage daily, every two days, or every three days starting
on the 3rd day and up to day 14. Mice in the control group received the same volume of PBS. Changes of body weight, DAI value, colon length, and colonic damage scores were monitored on day 14. For comparing the two formulations at 10 mg/kg daily and 40 mg/kg every three days at a similar total dose. 3% DSS-challenged mice were orally administered with 10 mg/kg MON-PEI or mesalazine daily, 40 mg/kg MON-PEI or mesalazine every three day starting on day 3. Survival rate, body weight and DAI changes of mice were monitored daily. The mice were sacrificed on day 14, and colon length and colonic damage scores of mice were determined. For examining the efficacy of MON-PEI in a setting of delayed therapy, 3% DSS-challenged mice were orally administered with 10 mg/kg MON-PEI or mesalazine daily, 40 mg/kg MON-PEI or mesalazine every three day starting on day 5. Survival rate, body weight and DAI changes of mice were monitored daily. The mice were sacrificed on day 21, and colon length and colonic damage scores of mice were determined.
Fig. S1. Serum cfDNA levels are not related to age or gender. Linear regression curves illustrating the correlation between serum cfDNA level and (A) gender and (B) age of IBD patients. Data are means ± SEM.
Fig. S2. Characterization of MON and MON-PEI. (A) Scanning electron microscopy image of MON. (B) TEM image of MON-PEI. (C) Nitrogen adsorption desorption isotherm and (D) pore size distribution of MON. (E) Nitrogen adsorption desorption isotherm and (F) pore size distribution of MON-PEI. (G) TGA curve of MON, PEI, and MON-PEI. (H) TEM image of MON after incubation in simulated gastric fluid (SGF) solution containing 100 μM H₂O₂ for 3 days. TEM image of (I) MON or (J) MON-PEI after incubation in SGF solution for 12 h. (K) Size and (L) Zeta potential of MON and MON-PEI under the same condition, respectively.
Fig. S3. Anti-inflammatory effects of MON-PEI in vitro. Viability of (A) RAW 264.7 macrophages and (B) HEK-TLR9 reporter cells treated with MON, PEI, and MON-PEI at various concentrations for 24 h. (C) Activation of HEK-TLR9 reporter cells via CpG in the absence or presence of MON, PEI, and MON-PEI for 24 h. The corresponding SEAP activity in supernatants from each group was determined with a QUANTI-Blue assay at OD$_{620}$. (D) RAW 264.7 macrophages were stimulated with CpG in the absence or presence of MON, PEI, and MON-PEI for 24 h. Supernatants were assayed for TNF-α via ELISA. (E) Relative fluorescence intensity of oxidized DCF in RAW 264.7 macrophages after incubation of different nanoscavengers in the presence or absence of LPS for 4 h. (F) Viability of RAW 264.7 macrophages were measured with SRB assay after overnight treatment with different nanoscavengers in the presence of 100 μM H$_2$O$_2$ for 24 h. (G) Activation of HEK-TLR4 reporter cells via LPS in the absence or presence of MON, PEI, and MON-PEI for 24 h. The corresponding SEAP activity in supernatants from each group was determined with a QUANTI-Blue assay at OD$_{620}$. (H) RAW 264.7 macrophages were stimulated with LPS in the absence or presence of MON, PEI, and MON-PEI for 24 h. Supernatants were assayed for TNF-α via ELISA. (I) Normalized concentrations of Na$_2$S$_4$ solution after treating MON or MON-PEI. (J) Viability of RAW 264.7 macrophages was measured with SRB assay after overnight treatment with different scavengers in the presence of 1.5 mM Na$_2$S$_4$ for 24 h. Data are means ± SEM (n=3 independent experiments; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S4. Oral gavage of MON-PEI prevents DSS-induced acute colitis in vivo. For the preventive setting via oral gavage, body weight, DAI, and serum inflammatory cytokine levels were determined, while colon images and H&E images of colonic section were captured on day 7. (A) Body weight and (B) DAI value of mice in each group were monitored. (C-F) Serum TNF-α, IL-6, IL-1β, and IL-4 levels were analyzed on days 1, 3, 5, and 7. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (G) Corresponding images of colons of indicated groups. Scale bar, 1 cm. (H) Colon sections of indicated groups via oral gavage were stained with H&E. Scale bar, 100 μm.
**Fig. S5.** Oral gavage of MON-PEI against DSS-induced acute colitis *in vivo*. For the therapeutic setting via oral gavage, colon images of colonic sections were captured on day 14. Scale bar, 1 cm.

| Water | 3% DSS |
|-------|--------|
| PBS   | PBS    | MON    | PEI    | MON-PEI |

![Colon images with scale bar, 1 cm](image)
Fig. S6. Intraperitoneal injection of MON-PEI against DSS-induced acute colitis in vivo. (A) Experimental scheme for DSS-induced acute colitis and administration of scavengers (MON, PEI, and MON-PEI) via IP injection. (B) Body weight, DAI, colon length, and colonic damage scores of mice in each group via oral gavage were determined at day 7. (C-G) For the therapeutic setting, daily (C) survival rate, (D) body weight, and (E) DAI changes of mice in each group were monitored. (F) Colon length and (G) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S7. Rectal enema of MON-PEI against DSS-induced acute colitis in vivo. (A) Experimental scheme for DSS-induced acute colitis and administration of scavengers (MON, PEI, and MON-PEI) via rectal enema. (B) Body weight, DAI, colon length, and colonic damage scores of mice in each group via rectal enema were determined on day 7. (C-G) For the therapeutic setting, daily (C) survival rate, (D) body weight, and (E) DAI value changes of mice in each group were monitored. (F) Colon length and (G) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S8. Comparison of the effect of scavengers on DSS-induced colitis via different administration routes. In the DSS-challenged model, scavengers (MON, PEI, and MON-PEI) were administrated via oral gavage, intraperitoneal (IP) injection, and rectal enema on day 3. (A-E) For the MON group, daily (A) survival rate, (B) body weight, and (C) DAI value changes of mice in each group were monitored. (D) Colon length and (E) colonic damage scores of mice of indicated groups were determined on day 14. (F-J) For the PEI group, daily (F) survival rate, (G) body weight, and (H) DAI changes of mice in each group were monitored. (I) Colon length and (J) colonic damage scores of mice of indicated groups were determined on day 14. (K-O) For the MON-PEI group, daily (K) survival rate, (L) body weight, and (M) DAI value changes of mice in each group were monitored. (N) Colon length and (O) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S9. Biosafety profiles of scavengers via oral gavage. Normal mice were treated daily with MON, PEI, or MON-PEI via oral gavage. (A) Body weight was monitored until 28 days after treatment. (B) ALT, (C) AST, (D) BUN, and (E) CRE were measured in serum on day 42. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (F) H&E staining of the heart, liver, spleen, lung, kidney, and colon was performed on day 42. Scale bar, 100 μm.
Fig. S10. Biosafety profiles of scavengers via intraperitoneal injection. Normal mice were treated daily with MON, PEI, or MON-PEI via IP injection. (A) Body weight was monitored until 28 days after treatment. (B) ALT, (C) AST, (D) BUN, and (E) CRE were measured in serum on day 42. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (F) H&E staining of the heart, liver, spleen, lung, kidney, and colon was performed on day 42. Scale bar, 100 μm.
Fig. S11. Biosafety profiles of scavengers via rectal enema. Normal mice were treated daily with MON, PEI, or MON-PEI via rectal enema. (A) Body weight was monitored until 28 days after treatment. (B) ALT, (C) AST, (D) BUN, and (E) CRE were measured in serum on day 42. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (F) H&E staining of the heart, liver, spleen, lung, kidney, and colon was performed on day 42. Scale bar, 100 μm.
| Water          | 2.5% TNBS            |
|---------------|----------------------|
| PBS           | PBS                  |
| PBS           | MON                  |
| MON           | PEI                  |
| PEI           | MON-PEI              |

**Fig. S12.** Rectal enema of MON-PEI against TNBS-induced chronic colitis *in vivo*. For the therapeutic setting via rectal enema, colon images of colonic sections were captured on day 5. Scale bar, 1 cm.
Fig. S13. Rectal enema of MON-PEI alleviates TNBS-induced chronic inflammation in vivo. In the same setting as in Fig. 4, serum (A) TNF-α, (B) IL-6, (C) IL-1β, and (D) IL-4 levels were analyzed on day 4. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S14. Oral gavage of MON-PEI ameliorates TNBS-induced chronic colitis in vivo. In the same setting as in Fig. 4, scavengers were administrated via oral gavage. (A) Daily body weight, (B) colon length and (C) colonic damage scores of mice in each group were monitored on day 5. (D) Corresponding images of colons of indicated groups. Scale bar, 1 cm. (E) Representative images of colon sections of indicated groups via oral gavage were stained with H&E. Scale bar, 100 μm. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S15. Intraperitoneal injection of MON-PEI ameliorates TNBS-induced chronic colitis in vivo. In the same setting as in Fig. 4, scavengers were administrated via IP injection. (A) Daily body weight, (B) colon length, and (C) colonic damage scores of mice in each group were monitored on day 5. (D) Corresponding images of colons of indicated groups. Scale bar, 1 cm. (E) Representative images of colon sections of indicated groups via oral gavage stained with H&E. Scale bar, 100 μm. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S16. Comparison of the effect of scavengers on TNBS-induced colitis via different administration routes. In the TNBS-challenged model, scavengers (MON, PEI) were administrated via oral gavage, IP injection, and rectal enema at day 1. (A-C) For the MON group, (A) daily body weight, (B) colon length and (C) colonic damage scores of mice in each group were monitored on day 5. (D-F) For the PEI group, (D) daily body weight, (E) colon length, and (F) colonic damage scores of mice in each group were monitored on day 5. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S17. Oral gavage of MON-PEI alleviates DSS-induced peritoneal inflammation in vivo. In the same setting as in Fig. 5. The colonic levels of (A) IL-10 and (B) IL-17 was analyzed on day 7 post-DSS challenge. Data are means ± SEM (n=6 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (C) The serum selenium level of mice in different groups on day 7 post-DSS challenge. (n=6 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (D) TLR9, MyD88, and p-p65 protein expression of peritoneal macrophages were determined by Western blotting. (E) TLR9 data are expressed as fold-change relative to the control group and normalized to GAPDH. (F) TNF-α gene expression of peritoneal macrophages was determined by real-time PCR. Data are expressed as fold-change relative to the PBS-treated normal group and normalized to GAPDH gene expression. Data are means ± SEM (n=3 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S18. Oral gavage of MON-PEI alleviates DSS-induced colonic inflammation in vivo. In the same setting as in Fig. 5, (A) Colon sections in each group were stained for TLR9, TNF-α, iNOS, F4/80, FOXP-3, and TGF-β. Scale bar, 100 μm. (B-E) Quantification of (B) TLR9, (C) TNF-α, (D) iNOS, (E) F4/80, (F) FOXP-3, and (G) TGF-β protein expression in (A). Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test).
Fig. S19. Biodistribution of MON-PEI. (A) Semi-quantitative analysis of ex vivo fluorescence images of the cecum from normal mice and DSS-challenged mice at 48 h after oral delivery of Cy7-labeled MON, PEI, and MON-PEI. (B) Semi-quantitative analysis of ex vivo fluorescence images of the cecum from normal mice and DSS-challenged mice at 72 h after oral delivery of Cy7-labeled MON, PEI, and MON-PEI. Data are means ± SEM (n=3 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (C) Near-infrared fluorescence images of heart, liver, spleen, lung, and kidney from normal and colitis mice at 2, 6, 12, 24, 48, and 72 h after oral delivery of Cy7-labeled MON, PEI and MON-PEI.
Fig. S20. Dose-dependent effect of MON-PEI on DSS-induced colitis via oral gavage. In the DSS-challenged model, MON-PEI were administrated with different dose via oral gavage. For the dose-dependent group, (A) body weight, (B) DAI, (C) colon length, and (D) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (E) Representative images of colon sections of indicated groups stained with H&E. Scale bar, 100 μm.
Fig. S21. Frequency-dependent effect of MON-PEI (10 mg/kg) on DSS-induced colitis via oral gavage. In the DSS-challenged model, MON-PEI were administrated with different dose frequency via oral gavage. For the frequency-dependent group with the same dose of 10 mg/kg, (A) body weight, (B) DAI value, (C) colon length, and (D) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (E) Representative images of colon sections of indicated groups stained with H&E. Scale bar, 100 μm.
Fig. S22. Frequency-dependent effect of MON-PEI (20 mg/kg) on DSS-induced colitis via oral gavage. In the DSS-challenged model, MON-PEI were administrated with different dose frequency via oral gavage. (A-D) For the frequency-dependent group with the same dose of 20 mg/kg, (A) body weight, (B) DAI, (C) colon length, and (D) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (E) Representative images of colon sections of indicated groups stained with H&E. Scale bar, 100 μm.
Fig. S23. Frequency-dependent effect of MON-PEI (40 mg/kg) on DSS-induced colitis via oral gavage. In the DSS-challenged model, MON-PEI were administrated with different dose frequency via oral gavage. For the frequency-dependent group with the same dose of 40 mg/kg, (A) body weight, (B) DAI, (C) colon length, and (D) colonic damage scores of mice of indicated groups were determined on day 14. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison tests). (E) Representative images of colon sections of indicated groups stained with H&E. Scale bar, 100 μm.
Fig. S24. Oral gavage of MON-PEI alleviates DSS-induced colonic damage in a delayed therapeutic setting. (A) In the same setting as in Fig. 6H, representative images of colon sections of indicated groups stained with H&E are shown. (B) In the same setting as Fig. 6M, representative images of colon sections of indicated groups stained with H&E are shown. Scale bar, 100 μm.
Fig. S25. Biosafety profiles of MON-PEI via different dose frequencies (frequency-dependent). Normal mice were treated with MON-PEI (40 mg/kg) daily, every two days or every three days via oral gavage for 14 days. (A) Body weight was monitored until 28 days after the last injection. (B) ALT, (C) AST, (D) BUN, and (E) CRE were measured in serum on day 42. Data are means ± SEM (n=6–8 mice per group; *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Tukey’s multiple comparison test). (F) H&E staining of the heart, liver, spleen, lung, kidney, stomach, and cecum was performed on day 42. Scale bar, 100 μm.
|                          | IBD    | HC    |
|--------------------------|--------|-------|
| Subjects, n              | 52     | 20    |
| Gender, female/male      | 29/23  | 12/8  |
| Median age, years(range) | 50.5(29-69) | 45.5(31-60) |
| Disease activity, n      |        |       |
| Active                   | 52     | -     |
| Inactive                 | 0      | -     |
| Mayo score               |        |       |
| Mild                     | 16     | -     |
| Moderate                 | 23     | -     |
| Severe                   | 13     | -     |
| Inflammation site        |        |       |
| Ileitis                  | 4      | -     |
| Colitis                  | 28     | -     |
| Colitis and ileitis      | 15     | -     |
| Proctitis                | 5      | -     |
| Medications, n           |        |       |
| Mesalazine               | 29     | -     |
| Corticosteroids          | 15     | -     |
| Thiopurines              | 2      | -     |
| Anti-TNF-α               | 6      | -     |
|                         | MON     | MON-PEI |
|-------------------------|---------|---------|
| Size (nm)               | 51 ± 3.6| 55 ± 4.3|
| Zeta potential (mV)     | -25.3 ± 1.8 | 46.7 ± 2.1 |
| Surface area (m²/g)     | 609.97  | 147.38  |
| Total pore volume (cm³/g)| 1.082   | 0.493   |
| Mass content of selenium (%) | 10.7  | 9.2     |
| Mass content of PEI (%)  | 0       | 15.50   |