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pH-triggered surface charge-reversal nanoparticles alleviate experimental murine colitis via selective accumulation in inflamed colon regions

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Abstract

In this study, we developed pH-triggered surface charge-reversal lipid nanoparticles (LNPs), loaded with budesonide, which could precisely deliver the drug to inflamed colon segments for the treatment of ulcerative colitis. Polyethyleneimine (PEI) was used to render LNPs cationic (PEI-LNPs), and Eudragit® S100 (ES) was coated on PEI-LNPs to obtain pH-triggered charge-reversal LNPs (ES-PEI-LNPs). ES coating avoided a burst drug release under acidic conditions mimicking the stomach and early small intestine environments and showed a sustained release in the colon. The surface charge of ES-PEI-LNPs switched from negative to positive under colonic conditions owing to pH-triggered removal of the ES coating. Bioimaging of the mouse gastrointestinal tract and confocal analysis of colon tissues revealed that ES-PEI-LNPs selectively accumulated in an inflamed colon. Furthermore, ES-PEI-LNPs mitigated experimental colitis in mice. These results suggest that the pH-triggered charge-reversal LNPs could be a promising drug carrier for ulcerative colitis therapy and other colon-targeted treatments.

Key words: Cationic lipid nanoparticles; Stimuli-triggered charge-reversal; Ulcerative colitis; Methacrylate copolymer; Budesonide

Ulcerative colitis (UC) is a major type of inflammatory bowel disease (IBD), an immune-mediated chronic or relapsing disorder of the gastrointestinal (GI) tract. IBD affects approximately 3.6 million people in the United States and Europe, and its incidence is alarmingly rising in previously low-incidence areas such as Asia.[1–3] UC is characterized by diffuse mucosal inflammation of the distal colon and rectum, with rectal bleeding, diarrhea, abdominal pain, and weight loss, which negatively affect the daily life.[4,5] The colectomy rate in 25% patients with acute sever UC increases with more than one hospital admission, reaching up to 40% after two subsequent admissions.[6] Since etiology of UC is unknown and there is currently no cure for UC, current therapeutic strategies are aimed to induce the remission, prevent inflammatory episodes, and ensure colectomy-free survival.

Colon-specific oral drug delivery is an active area of research for the therapy of IBD since it enables localized treatment, which improves the efficacy of therapeutics and reduces systemic toxicities.[7,8] However, precise delivery of a drug to target inflamed segments of the colon still remains a problem. Since inflammation occurs only in limited sectors of colonic tissue, varying from patient to patient, a substantial amount of a drug can be unintentionally delivered to non-inflamed healthy regions. This misallocation of the drug is hardly avoidable upon the use of

Abbreviations: DiR, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide; DSS, dextran sulfate sodium; ES, Eudragit® S100; GI, gastrointestinal; H&E, hematoxylin and eosin; HPLC, high-performance liquid chromatography; IBD, inflammatory bowel disease; ICR, imprinting control region; IVIS, *in vivo* imaging system; LNP, lipid nanoparticle; MPO, myeloperoxidase; PDI, polydispersity index; PEI, polyethyleneimine; TEM, transmission electron microscope; TNF-α, tumor necrosis factor-alpha; UC, ulcerative colitis.

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conventional colon-specific drug delivery systems, often resulting in undesirable adverse effects and therapy failure. Currently marketed delivery systems such as Budenofalk® and Entocort® appear to show poor selectivity for inflammation.[9–11] Thus, an ideal colon-specific oral drug delivery system should have the ability to deliver a drug selectively and exclusively to inflamed regions to achieve maximum therapeutic outcomes with minimized systemic side effects.

In this regard, nanotechnology-based colon-targeted oral delivery has recently gained much attention owing to the ability of designed nanoparticles to accumulate in inflamed colon tissue. The accumulation of NPs in the inflamed area is attributed to disrupted intestinal barrier functions, a highly increased number of immune-related cells, which take up particles, and elevated levels of mucus production.[12] Several studies have demonstrated that NPs exhibit size-dependent adhesion to inflamed tissues of the colon, combined with an enhanced therapeutic effect in experimental colitis.[13–17] For instance, our group[15,18] and Ali H et al[19] reported that drug-loaded nano-sized polymeric carriers mitigated colitis by accumulation in the inflamed colon. Besides the size dependency, surface properties of NPs also play a crucial role in the selective accumulation in an inflamed area. Recent studies have suggested that the cationic property of NPs can enhance mucoadhesion to inflamed tissues in the colon and subsequent uptake by inflammatory cells.[13,20–22] However, non-specific electrostatic binding of positive surface charge NPs to the mucosal membrane of the upper GI tract, especially the small intestine, can compromise the advantages of cationic NPs in selective delivery to an inflamed colon.[23–26] An intelligent approach that includes shielding of the cationic surface charge of NPs before they reach the colon and subsequent deshielding in the colon can be employed to overcome the cationic surface challenge in oral colon-targeted delivery.

Among advanced drug delivery nanocarriers, lipid NPs (LNPs) are a promising colloidal carrier system for oral delivery showing a number of advantages such as controlled drug release characteristics, surface modification, no carrier biotoxicity, no organic solvents, and the feasibility of large-scale production.[27–29] However, a premature burst drug release in the stomach and small intestine and instability under acidic conditions are the major limitations for the clinical use of LNPs in colon-targeted drug delivery. Cationic LNPs have been prepared using polyelectrolyte complexes and showed an improved stability under acidic conditions, as well as improved mucoadhesion and cellular uptake.[30] However, the cationic polymer layer may not be able to protect from an unwanted drug release under acidic conditions of the stomach and small intestine. An additional, pH-sensitive polymer layer could be used to prevent the drug release in the early parts of the GI tract. The layer would be dissolved at colonic pH to expose the cationic surface of LNPs and allow their increased accumulation in the inflamed colon.

Based on these considerations, we report herein the intelligent design of LNPs with negative-to-positive surface charge reversal and controlled drug release characteristics, which are both triggered by the pH in the colon (Figure 1). Budesonide, a corticosteroid drug clinically used in UC therapy,[31] was encapsulated in the LNP formulations. The size, zeta potential, shape, and drug-loading capacity of the LNP formulations were characterized. Drug release profiles were evaluated in different pH environments resembling those of the GI tract. After physicochemical characterization, the accumulation in an inflamed colon and the *in vivo* therapeutic efficacy of the LNP formulations were evaluated in dextran sulfate sodium (DSS)-induced acute colitis mice.

Methods

Sources for materials and some methods are shown in the Supporting information.

Preparation of LNP formulations

LNPs were prepared by the hot homogenization method[32] with some modifications. Briefly, 100 mg of Compritol, 50 mg of Phospholipon 90 G, and 10 mg of budesonide were melted in 1 mL of ethyl acetate to obtain a transparent solution. An aqueous surfactant phase was prepared by dissolving 200 μL of Tween 80 in 10 mL of distilled water, followed by heating to the temperature of the lipid phase. The hot aqueous phase was dispersed in the lipid phase and homogenized at 20,000 rpm for 5 min. The resulting coarse emulsion was ultrasonicated at a 90% amplitude for 5 min using a probe sonicator. The solvent was evaporated in an ice bath and LNPs were collected by centrifugation. PEI-LNPs were prepared using the same technique, with a slight modification. Briefly, a 0.25% PEI solution was prepared in 10 mL of distilled water containing 200 μL of Tween 80 and used as an external phase. Then, the prepared PEI-LNPs were incubated for 1 h with a gentle shaking in an anionic solution of ES in phosphate-buffered saline (PBS), at a 1:4 core to coat ratio, to produce ES-PEI-LNPs (Figure 1, *A*).

In vivo imaging

To evaluate the colon-specific drug delivery potential of an LNP formulation after oral administration, an *in vivo* imaging system (IVIS) (FOBI; Neoscience, Suwon, Korea) was used. The near-infrared dye DiR was loaded as a fluorescent probe into the LNP formulations. Healthy and DSS-induced colitis ICR mice, fasted for 24 h, were orally administered the DiR-loaded LNP formulations at an equivalent DiR concentration (0.5 mg DiR/kg). Blank LNP formulations were also used. The mice were sacrificed 12 h after the administration, and the abdominal skin was removed to analyze the GI tract for the distribution of the LNP formulations.

Accumulation of LNP formulations in an inflamed colon

The hydrophobic fluorescent marker C-6 was loaded into the LNP formulations to facilitate their detection by confocal microscopy and quantification in sections of healthy and inflamed colon tissues after 12 h of oral administration. Blank NPs without C-6 were also tested. For confocal microscopy, a colon specimen was embedded in Tissue-Tek® optimum cutting temperature compound and cut into 5-μm sections using a cryostat (Reichert, Germany). For the quantitative study, the colon samples were freeze-dried and homogenized into powder. Subsequently, 30 mg of the powder was used to extract C-6 with 1 mL of chloroform/methanol (1:1, v/v), and the C-6 extracted was quantified using a

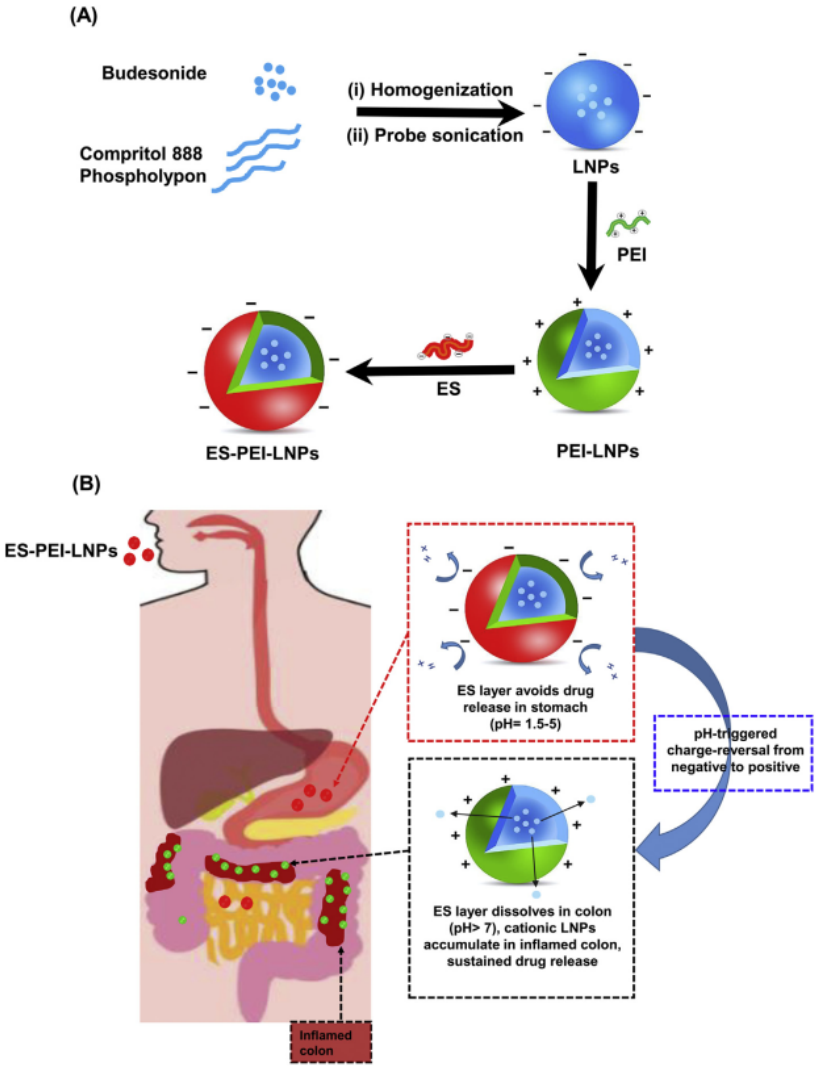

Figure 1. Schematic illustration of the LNP formulations preparation process and proposed mechanism of the drug release in the GI tract. (**A**) The fabrication process of LNP formulations; (**B**) proposed pH-triggered drug release and surface charge conversion of ES-PEI-LNPs.

fluorescence plate reader (triStar LB 941, Berthold Technologies, Bad Wildbad, Germany).

Drug treatment

After induction of colitis, DSS water was replaced with normal tap water, and drug treatment was started. Each treated group (LNPs, PEI-LNPs, and ES-PEI-LNPs treatment) received an equal dose of budesonide (0.168 mg/kg) once a day for 5 days in the form of particles suspended in water, which were administered by oral gavage under isoflurane anesthesia. Blank NPs (LNPs, PEI-LNPs, and ES-PEI-LNPs without budesonide) were also tested. The healthy control and colitis control groups received no drug treatment.

Histological analysis

Small segments of the mice colon from each study group were fixed in 10% formalin for 24 h at room temperature and then embedded in paraffin for histological examinations. Tissues were sectioned at 5-μm thickness with a microtome (Reichert, Germany) and stained with hematoxylin/eosin (H&E). Images were acquired by light microscopy (Zeiss, Axioskop, Germany). The degree of inflammation and epithelial injury on microscopic cross-sections of the colon was graded semi-quantitatively from 0 to 4.[33]

Table 1
Physicochemical characteristics of LNP formulations.

Formulations	Particle size (nm)	Zeta potential (nm)	Polydispersity index	Drug loading efficiency (%)	Encapsulation efficiency (%)
LNPs	211 ± 17	−12 ± 3.8	0.27 ± 0.03	5.9 ± 0.2	64 ± 2.5
PEI-LNPs	243 ± 11	+23 ± 5.6	0.23 ± 0.02	6.0 ± 0.2	63 ± 3.0
ES-PEI-LNPs	302 ± 13	−30 ± 5.3	0.19 ± 0.02	4.8 ± 0.4	58 ± 5.2

Results are expressed as the mean±SD (n=3).

Myeloperoxidase assay and determination of pro-inflammatory cytokines levels in the colon tissue

Myeloperoxidase (MPO) activity was measured according to an established method.[34] Briefly, distal colon specimens were homogenized in 1 mL of pre-chilled potassium phosphate buffer (50 mM K_2HPO_4 and 50 mM KH_2PO_4, pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) followed by sonication for 10 s, three freeze–thaw cycles, and centrifugation at 14,000 rpm at 4 °C for 3 min. The clarified supernatants (0.1 mL) were added to 2.9 mL of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/mL *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide, and the change in absorbance at 460 nm was measured using a UV spectrophotometer (Shimadzu) for 5 min at 25 °C. Concentrations of the tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in colon tissue of the untreated and treated mice were determined by a sandwich-type enzyme-linked immunosorbent assay (ELISA) using kits (Biolegend, San Diego, CA, USA; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Results

Preparation and characterization of LNP formulations

Two oppositely charged polyelectrolytes, cationic PEI and anionic ES, were directly assembled on LNPs to prepare pH-triggered drug-releasing and charge-reversal LNPs (ES-PEI-LNPs) as shown in Figure 1, *A*. The net negative surface charge (−12 mV) of LNPs (Table 1) facilitated the alternate deposition of the polyelectrolytes via an electrostatic interaction (Figure 2, *A*). The addition of PEI resulted in the surface charge shift from negative (−12 mV) to positive (+23 mV) and a slight increase in the particle size, from 211 to 243 nm (Figure 2, *B* and Table 1). The addition of anionic ES as a pH-sensitive layer resulted in the alteration of the surface charge to negative (−30 mV), as well as an increase in the particle size (302 nm) compared with that of PEI-LNPs (243 nm), indicating successful fabrication of ES-PEI-LNPs. The PDI values of the LNP formulations were smaller than 0.3, showing a narrow distribution of the particle size.[35] The morphology of LNPs, PEI-LNPs, and ES-PEI-LNPs was observed under TEM as shown in Figure 2, *D*. All LNP formulations exhibited a spherical particle shape without cracking and aggregation. The drug-loading and encapsulation efficiencies of the LNP formulations are shown in Table 1. The encapsulation efficiency of ES-PEI-LNPs was slightly lower than that of LNPs and PEI-LNPs, which might be due to a leakage of the encapsulated drug during the addition process of the ES layer.

pH-dependent in vitro drug release and zeta potential

LNPs and PEI-LNPs showed an initial burst drug release (over 40% in the first 1 h) under the stomach condition (pH 1.2), followed by a sustained release under the condition mimicking that in the upper part of the small intestine (Figure 3, *A*). Approximately 60% of budesonide was released from LNPs and PEI-LNPs in the first 6 h. On the other hand, ES-PEI-LNPs released only 15.9% of the drug in the first 6 h (7.9% under the stomach condition), proving their ability to prevent an unnecessary drug release in the upper GI tract. At the terminal ileum pH (pH 7.4), the drug release from ES-PEI-LNPs was much faster than that at the acidic pH values because of the dissolution of the ES layer. The pH-dependent dissolution of ES-PEI-LNPs was also confirmed by measuring the surface charge under the acidic and alkaline conditions as shown in Figure 3, *B*. ES-PEI-LNPs maintained the negative surface charge under the acidic conditions (pH 1.2 and 6.8) owing to the presence of the ES layer, whereas the surface charge changed to positive after incubation under the alkaline pH (7.4) owing to the dissolution of ES and the exposition of the cationic PEI-LNPs.

Targeted delivery to inflamed colon tissue

The targeting ability of the LNP formulations for the site of inflammation in the colon was examined using IVIS and confocal microscopy. As shown in Figure 4, *A*, LNPs showed fluorescence in the stomach and jejunum areas, which might be due to LNP aggregation, owing to their instability under acidic conditions, as reported previously.[30] PEI-LNPs were distributed in the small intestine, indicating that the PEI coating provided stability under acidic conditions and helped avoid aggregation. However, the burst drug release and positive surface charge-mediated mucoadhesion might have resulted in the retention of PEI-LNPs in the small intestine. In contrast, the colon of the ES-PEI-LNP-administered mice exhibited a higher fluorescence than that detected in the colons of the LNP- and PEI-LNP-administered mice. Importantly, an even higher fluorescence was observed in the colon of the ES-PEI-LNP-treated colitis mice than that in the colons of the ES-PEI-LNP-treated healthy mice, demonstrating the accumulation of LNPs in inflamed areas of the colon. Next, we evaluated the accumulation of C-6-loaded LNP formulations in healthy and inflamed colon tissues by confocal microscopy. As shown in Figure 4, *B*, LNPs and PEI-LNPs showed a negligible fluorescence intensity in healthy and inflamed colon tissues, whereas ES-PEI-LNPs spread over the healthy colon tissue and substantially accumulated in inflamed segments of the colon. The C-6 concentration in inflamed colon tissues was six-fold higher than

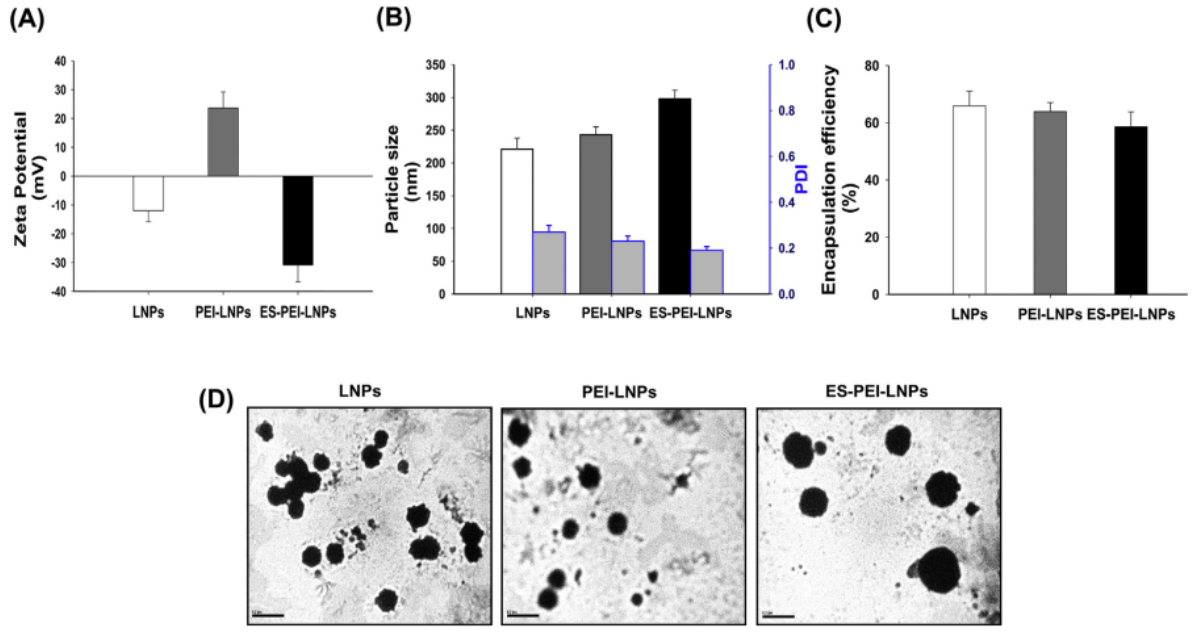

Figure 2. Characterization of LNP formulations: zeta potential (**A**); zeta size and PDI (**B**); drug encapsulation efficiency (**C**); and TEM images of the LNP formulations (**D**).

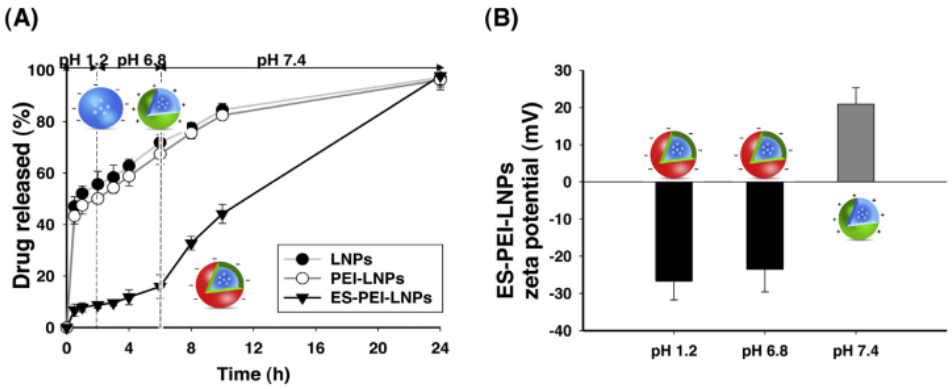

Figure 3. pH-dependent *in vitro* drug release from LNP formulations (**A**); and pH-triggered surface charge conversion of ES-PEI-LNPs (**B**).

that in healthy colon tissue after administration of ES-PEI-LNPs (Figure 4, *C*). The colon tissue sections from the blank LNP formulations administered group did not show fluorescence and the data are not shown in the results.

In vivo therapeutic efficacy

Body weight changes, diarrhea, and bleeding scores

The body weight changes, stool consistency, and rectal bleeding scores are shown in Figure 5. A faster recovery of the body weight was observed in the ES-PEI-LNP-treated group compared to that in the untreated and other treated colitis groups (Figure 5, *A*). Furthermore, the ES-PEI-LNP-treated group showed the most prominent reduction in diarrhea and the bleeding score (Figure 5, *B*, *C*). Blank NPs did not show a reduction in clinical parameters of colitis (data not shown).

Macroscopic assessment of colitis after treatment

All mice were sacrificed after 5 days of drug treatment. The colons were isolated from the mice and representative photographs are shown in Figure 5, *D*. The healthy mice showed a colon length of 11.4 ± 0.63 cm; however, a significant reduction in the colon length was observed after the induction of DSS-mediated colitis, which resulted in the colon length of 5.8 ± 0.62 cm. The mice

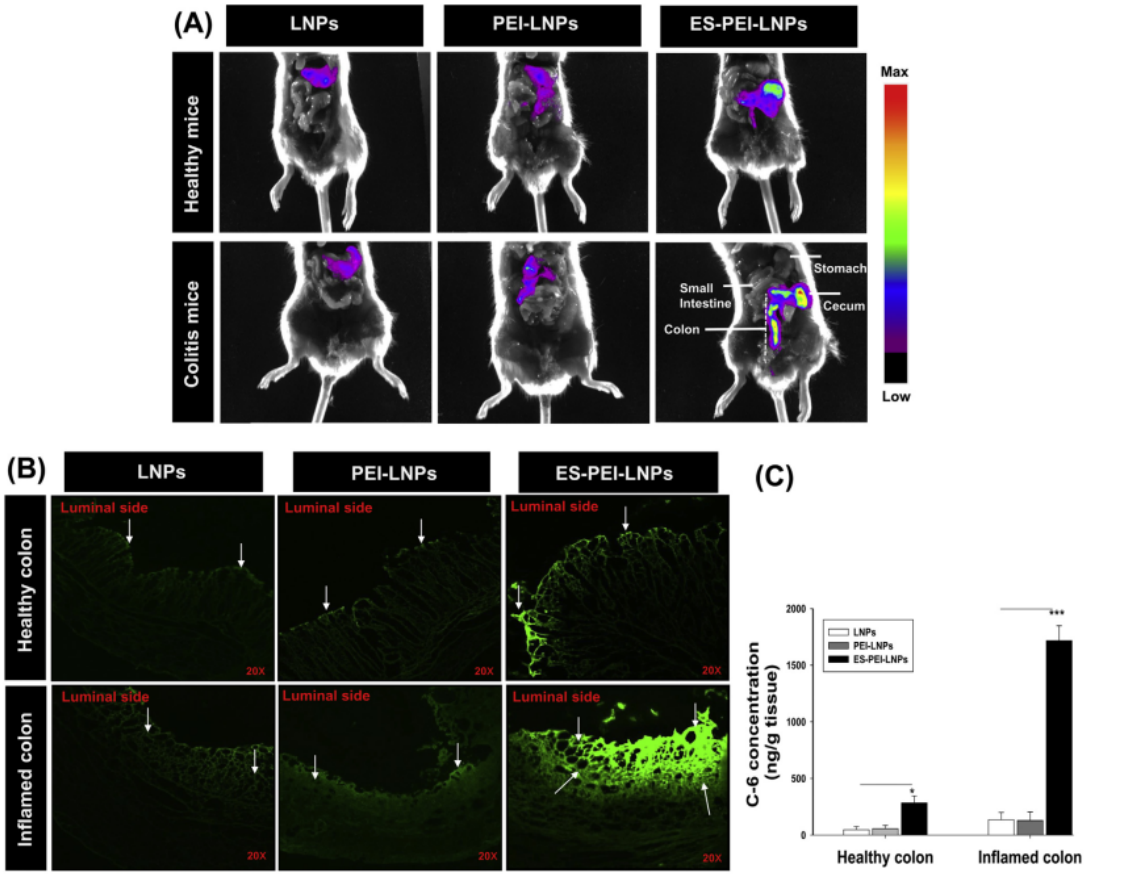

Figure 4. *In vivo* localization of LNP formulations in the GI tract of mice 12 h after oral administration. (**A**) Localization of DiR-loaded LNP formulations in the GI tract of healthy and colitis mice; (**B**) confocal images of healthy and inflamed colon cross-sections from mice administered C-6-loaded LNP formulations; (**C**) quantitative determination of C-6 in healthy and inflamed colon tissue. Data are presented as the mean±SD (n=3 per group; *P<0.05, ***P<0.001).

treated with the LNP formulations showed an increase in the colon length compared to that in the colitis control. In particular, the colon length increased to 10 ± 0.91 cm in the ES-PEI-LNP-treated mice, which was much closer to the baseline than what was observed in the remaining colitis groups. Administration of DSS increased the spleen weight, which is one of the classic colitis symptoms (Figure 5, *E*). Indeed, the spleen weight was markedly lower in the ES-PEI-LNP-treated group than that in the colitis control group and other treated groups. These results demonstrated that ES-PEI-LNPs ameliorated colitis to a greater extent than LNPs and PEI-LNPs did. Blank LNP formulations administered group did not show improvement in colitis and data were excluded from the results.

Histological assessment of colitis

Histological effects of LNPs, PEI-LNPs, and ES-PEI-LNPs on DSS-induced colitis were examined by H&E staining (Figure 6, *A*). The DSS-treated mice exhibited an epithelial erosion, loss of goblet cells, interstitial edema, and a general increase in the number of inflammatory cells infiltrated in the lamina propria, all of which are obvious signs of inflammation. Tissue damage was also observed in the LNP- and PEI-LNP-treated mice groups. Interestingly, the ES-PEI-LNP-treated mouse group demonstrated morphological tissue structures resembling those of healthy tissues, indicating epithelial restoration and a decrease in cell infiltration. Histological scoring of the tissue sections confirmed that inflammation was significantly alleviated (P<0.001) in the ES-PEI-LNP-treated group, as compared to that in the other treated groups (Figure 6, *B*).

Immunostaining

The severity of inflammation in LNP formulation-treated and untreated mouse colon tissues was further evaluated microscopically using E-cadherin immunofluorescence staining. E-cadherin is a major component of adherens junctions which plays a crucial role in epithelial cell–cell adhesion, as well as in the maintenance of tissue architecture. Impaired expression of E-cadherin has been linked to disturbed intestinal barrier function and homeostasis.[36] As shown in Figure 7, the low level of E-cadherin expression in untreated colitis mouse tissue confirmed the severity of inflammation induced by DSS intake. The drug-treated mouse groups revealed that the E-cadherin expression in colonic epithelial cells dramatically increased in

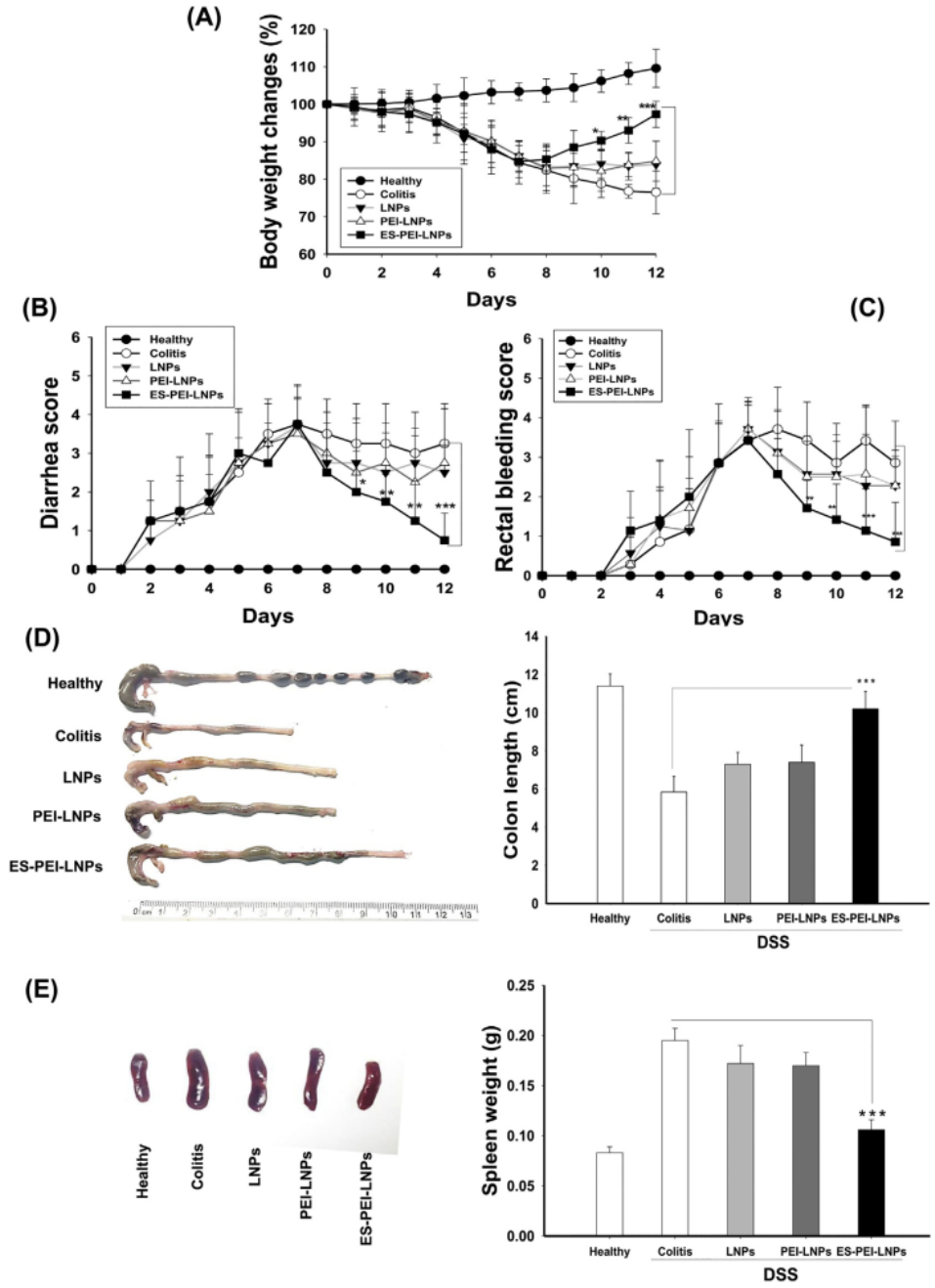

Figure 5. Clinical parameters evaluated during the entire experimental period of colitis treatment: (**A**) % body weight changes; (**B**) diarrhea scores; (**C**) rectal bleeding scores; (**D**) colon photographs and lengths (cm); (**E**) spleen photographs and weights (g). n=5 per group; *P<0.05, **P<0.01, ***P<0.001 compared with the colitis control.

the ES-PEI-LNP-treated mice but not in the mice treated with LNPs and PEI-LNPs when compared with that in the untreated colitis mice. The E-cadherin expression results corroborate the observations from the H&E staining study, shown in Figure 6, *A*.

MPO activity and pro-inflammatory cytokines levels in colon tissue

We also measured the colonic MPO activity (Figure 8, *A*) and levels of TNF-α (Figure 8, *B*) as an indicator of the extent of

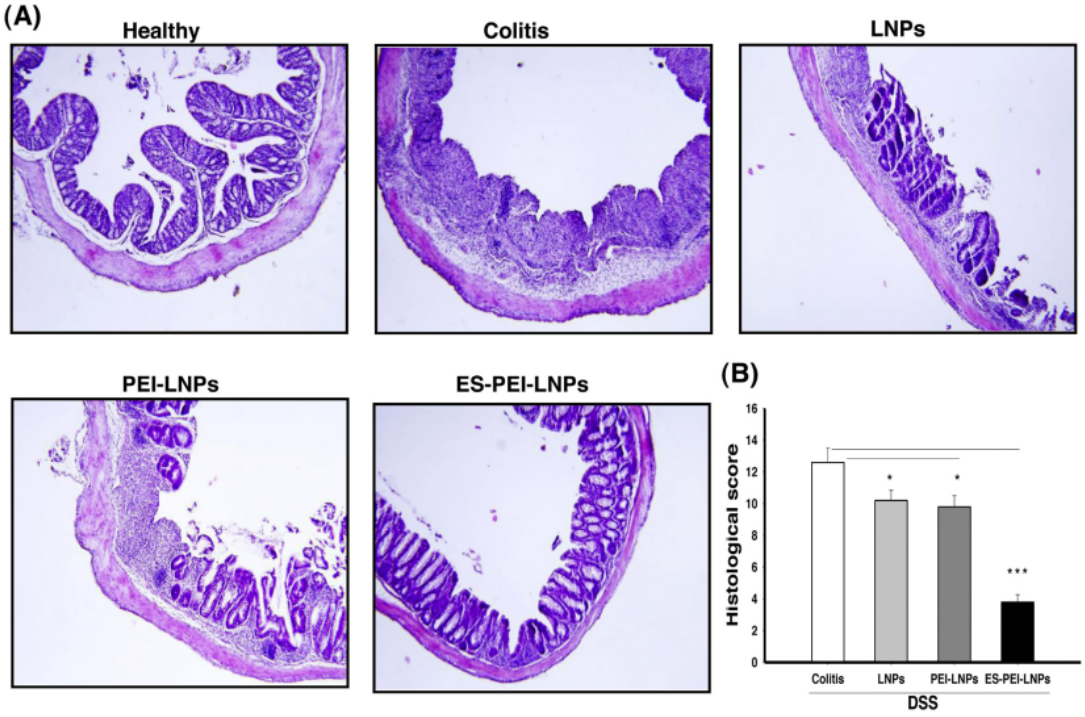

Figure 6. Histological evaluation of colon tissue from the colitis control and budesonide-treated groups. (**A**) H&E staining of colon sections (5 μm thick). Images of tissues are shown at 100× magnification. (**B**) Histological scores. Data are presented as the mean±SD (n=5 per group). *P<0.05, ***P<0.001 compared with the colitis control.

neutrophil and macrophage infiltration, respectively. All animals suffering from colitis exhibited an elevated MPO activity TNF-α and IL-6 expression, as compared to those in the healthy mice. However, the MPO activity and pro-inflammatory cytokines levels were substantially lower in the LNP formulation-treated groups, as compared to those in the untreated colitis group. It is worth noting that the MPO activity and cytokine concentrations were significantly lower in the ES-PEI-LNP-treated group (P<0.001) than those in the other groups.

Discussion

In recent years, cationic LNPs have gained much attention as an oral delivery system with enhanced intestinal drug absorption owing to the mucoadhesive property via an electrostatic interaction with mucin.[24,30,37,38] These properties of cationic LNPs are desirable for selective accumulation in inflamed colonic tissues, which requires mucoadhesion and uptake by inflammatory cells. However, the cationic property is not preferred in the upper GI tract because it leads to non-specific binding of LNPs to the mucosa before they reach the colon, which may negatively affect the targeted delivery to the colon. Besides the binding problem, an unwanted initial burst drug release in the upper GI tract is another intrinsic problem of LNPs,[39] especially those designed for colon-specific delivery. To simultaneously solve the problems, we hypothesized that the reinforcement of cationic LNPs with a pH-sensitive, enteric, and anionic polymer (i.e., ES) would result in an intelligent nanocarrier system, providing a pH-triggered drug release and charge reversal from anionic to cationic characteristics in the target area. In the present study, we prepared budesonide loaded three LNP formulations, namely, LNPs, PEI-LNPs, and ES-PEI-LNPs and their drug release characteristics, targeted accumulation, and therapeutic potential for UC therapy were evaluated. Budesonide is a synthetic glucocorticoid which interacts with intracellular glucocorticoid receptor and effectively inhibits pro-inflammatory cytokines secretion by inflammatory cells such as macrophages and T-lymphocytes, and thus is clinically used for the treatment of UC with the colon-specific formulations.[40,41] It is worth noting that oral administration of free budesonide without a colon delivery system did not show any alleviation of experimentally-induced murine colitis.[15]

Anionic LNPs were prepared from Compritol using a hot homogenization method and probe sonication to break down large lipid spheres into nano-scale particles.[32] Compritol is a non-toxic lipid composite belonging to Generally Recognized As Safe (GRAS) status and FDA inactive ingredient databases with a long history of use in cosmetics, foods, and pharmaceutical industry.[42] Moreover, LNPs have been used in marketed cosmetics products.[43] However, the safety of LNPs has not been fully investigated for colitis applications and further studies are warranted. Cationic LNPs were prepared by coating the surface of LNPs with PEI through electrostatic interaction (PEI-LNPs). The high positive charge of PEI leads to electrostatic interactions with negatively charged biological

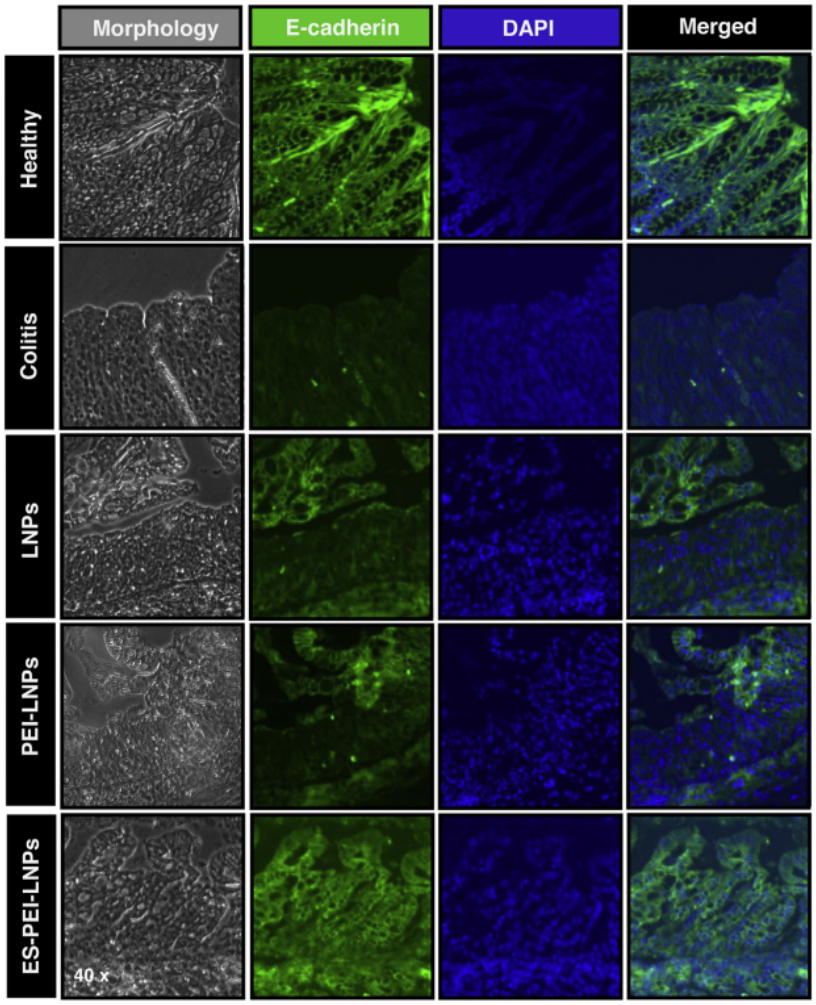

Figure 7. E-cadherin immunostaining of colon tissue from the healthy, colitis, and LNP formulation-treated mouse groups.

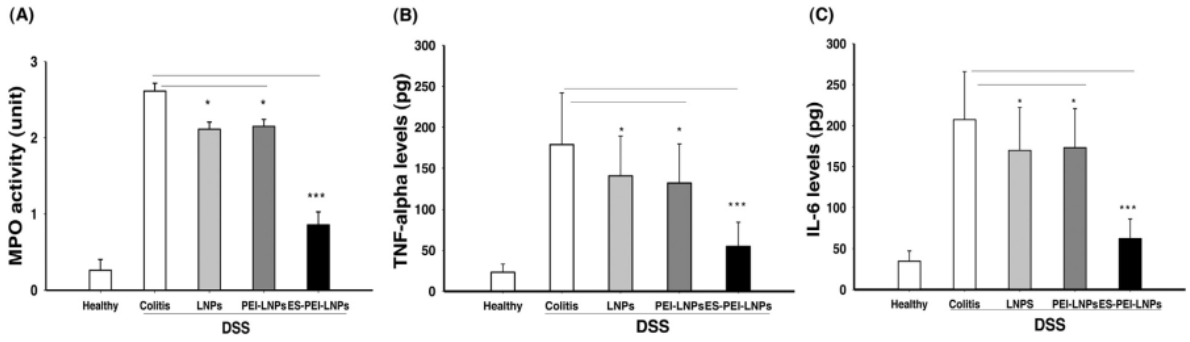

Figure 8. Average MPO activity and pro-inflammatory cytokines expression in the healthy control group, colitis control group, and drug-loaded LNP formulation-treated-groups: MPO assay (**A**); TNF-α levels (pg) (**B**) and IL-6 levels (pg). (*P<0.05, ***P<0.001).

molecules and thus facilitates the accumulation of NPs in tissues.[44] In a recent study, PEI has been used as an outer shell of NPs to enhance their accumulation in DSS-induced acute colitis areas.[20] However, PEI-LNPs, similar to LNPs, are still not suitable for colon-targeted delivery because of the premature burst drug release in the stomach and early small intestine. As shown in the *in vitro* release study (Figure 3, *A*), LNPs and PEI-LNPs exhibited a burst drug release, with over 60% of the drug released in an acidic pH medium mimicking the stomach and early small intestine conditions. To minimize the burst drug release in an acidic environment of the stomach and early small intestine, we chose an anionic methacrylic acid copolymer, ES, as the final coating material with a pH-responsive enteric property. It is reported that colon pH significantly varies in active UC patients from pH 5.5 to as low as 2.3 when compared to healthy individual colon pH which ranges from 6.8 to 7.2.[17] However, ileum pH in UC patients was reported in normal range from pH 7.2 to 8.3.[45] Based on these pH values in UC patients, we proposed that ES coating of ES-PEI-LNPs would dissolve at ileum pH and allow a sustained drug release hereafter in the colon. ES-PEI-LNPs avoided the burst drug release at acidic pH, but allowed a controlled release under ileum- and colonic-like conditions (Figure 3, *A*). These results demonstrated that ES-PEI-LNPs are able to effectively retain the entrapped drug during their transit from the stomach until reaching the colon.

Unlike the previously reported pH-triggered colon-specific delivery polymeric NPs and pH-independent lipid-based NPs whose main purpose was merely the protection of early burst drug release in the stomach and the upper small intestine,[18,19,46] and the controlled release,[47] respectively, ES-PEI-LNPs were designed for smart conversion of surface charge (from anionic to cationic), which not only prevents early burst drug release in the upper GI tract, but avoids unwanted mucus adhesion prior to reaching the colon, followed by enhanced accumulation of NPs and a sustained drug release in the target inflamed regions. We assumed that the unique pH-triggered surface charge reversal property would result in enhanced accumulation in the inflamed colon and hence improved therapeutic efficacy.[22] The surface charge reversal property of ES-PEI-LNPs was tested in media mimicking the stomach, small intestine, and colon pH conditions. ES-PEI-LNPs maintained a negative surface charge in an acidic medium and switched to a positive surface charge in a colon pH medium by the dissolution of the negatively charged ES layer (Figure 3, *B*).

Next, we evaluated the *in vivo* drug delivery potential and accumulation of ES-PEI-LNPs in an inflamed colon using IVIS and confocal microscopy. *In vivo* imaging of the mouse GI tract and confocal microscopy images of colon tissue revealed the greater accumulation of fluorescent ES-PEI-LNPs in inflamed colon tissue compared to that in healthy colon tissue (Figure 4). On the other hand, LNPs and PEI-LNPs showed a low fluorescence intensity in the healthy and inflamed colon. On the one hand, these results can be attributed to different release patterns between LNPs, PEI-LNPs, and ES-PEI-LNPs. On the other hand, the ES layer would dissolve at the colonic pH, exposing the cationic PEI-LNPs, which are expected to accumulate in inflamed colon segments owing to the nano size and enhanced cellular uptake and mucoadhesive characteristics.[48] These characteristics of ES-PEI-LNPs make it a promising delivery system for UC therapy when compared to pH-dependent conventional formulations such as tablets, capsules, and pellets which are subjected to diarrhea, which results in decreased GI tract transit time and therefore decreases in therapeutic outcomes.[12] Taken together, ES-PEI-LNPs preferentially adhere to the inflamed colon mucosa and create a reservoir of an encapsulated drug at the site of inflammation, thereby prolonging the local drug availability.

After assessing the *in vitro* and *in vivo* colon targeted drug delivery potential, the therapeutic efficacy of budesonide loaded LNP formulations was evaluated in a DSS-induced colitis mice. The DSS-induced colitis model was chosen because it exhibits certain characteristics similar to those present in human UC.[49] Among the LNP formulations, ES-PEI-LNPs exhibited the highest colitis-mitigating effect. The ES-PEI-LNP-treated mice showed a higher body weight and colon length and lower spleen weight, as well as reduced diarrhea and bleeding scores, indicating a reduction in inflammation owing to the improved drug delivery potential of ES-PEI-LNPs for the inflamed colon (Figure 5). Further, the general microscopic appearance of the mice treated with ES-PEI-LNPs was quite similar to that of the healthy control group, with epithelium recovery and no severe infiltration of inflammatory cells (Figure 6). The E-cadherin immunostaining results further supported the findings from H&E tissue staining. The increased expression of E-cadherin in epithelial cells of the colon tissue treated with ES-PEI-LNPs indicated re-epithelization and amelioration of inflammation (Figure 7). Furthermore, the ES-PEI-LNP-treated group showed significantly lower MPO levels than those in the other treatment groups (Figure 8, *A*). MPO is the most abundant protein in neutrophils and is widely used in IBD animal models and in feces of IBD patients as a standard indicator of colitis.[50] In addition, ES-PEI-LNPs suppressed proinflammatory cytokines more significantly than LNPs and PEI-LNPs did (Figure 8, *B*). Taken together, MPO levels and the expression of pro-inflammatory cytokines were significantly reduced in colon tissues of the mice treated with ES-PEI-LNPs when compared to colitis mice and LNPs and PEI-LNPs treated mice, which further support the enhanced drug delivery and therapeutic potential of ES-PEI-LNPs for the treatment of UC. The blank NPs (without budesonide) group did not show any therapeutic effects in all *in vivo* experiments (data not shown), indicating that the blank carriers have no influence on therapeutic effects of budesonide-loaded carriers.

In conclusion, pH-triggered surface charge-reversal LNPs (ES-PEI-LNPs), loaded with budesonide, were successfully prepared by polyelectrolyte complexation. LNPs and PEI-LNPs showed an unwanted burst drug release in a buffered medium mimicking the stomach and small intestine pH, whereas the reinforcement of PEI-LNPS with ES and the formation of the enteric outer layer provided sufficient protection for the loaded drug in an acidic environment and a pH-responsive drug release in a colonic pH medium. Further, the pH-triggered charge reversal of ES-PEI-LNPs facilitated their accumulation in an inflamed colon and markedly enhanced the therapeutic outcomes in a DSS-induced colitis mouse model. Therefore, we conclude that the surface charge-reversal smart ES-PEI-LNPs described in this study have a promising potential for targeted drug delivery to the inflamed colon in the therapy of UC.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nano.2018.01.003.

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