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Oral delivery of curcumin *via* porous polymeric nanoparticles for effective ulcerative colitis therapy†

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Abstract

Oral drug delivery has been considered as a promising strategy for ulcerative colitis (UC) therapy. Here, an emulsion solvent evaporation technique was employed to prepare non-porous curcumin (CUR)-loaded polymeric nanoparticles (NPs) and porous CUR-loaded polymeric NPs in the absence or presence of ammonium bicarbonate. The resultant CUR-loaded NPs (non-porous NPs and porous NPs) had a desirable mean particle size of around 260 nm with a narrow size distribution, a uniform pore size distribution, slightly negative-charged surface, high encapsulation efficiency and controlled drug release capacity. *In vitro* experiments indicated that Raw 264.7 macrophages exhibited time-dependent accumulation profiles of NPs during the initial 2 h of co-incubation. Furthermore, we found that porous NPs inhibited the secretion of the main pro-inflammatory cytokines (TNF- α , IL-6 and IL-12) and the production of reactive oxygen species much more efficiently than non-porous NPs. Most importantly, *in vivo* studies demonstrated that oral administered porous NPs had a superior therapeutic efficiency in alleviating UC compared with non-porous NPs. The results collectively suggest that porous polymeric NPs can be exploited as efficient oral drug carriers for UC treatment.

Graphical abstract

Curcumin (CUR) has been encapsulated into porous polymeric nanoparticles (NPs), and the resultant CUR-loaded porous polymeric NPs exhibited a superior therapeutic efficacy in alleviating Ulcerative colitis, in comparison with CUR-loaded non-porous polymeric NPs.

[†]Electronic supplementary information (ESI) available: *In vitro* cytotoxicity of non-porous NPs and porous NPs against Raw 264.7 macrophages (a) and CT-26 cells (b). See DOI: 10.1039/b000000x/

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1. Introduction

Ulcerative colitis (UC), one of two major forms of inflammatory bowel diseases, is characterized by several typical symptoms such as diarrhea, mucosal ulceration and hemafecia.^{1, 2} In recent years, the incidence of UC is rapidly increasing worldwide, especially in many developing countries.³ Although a lot of efforts have been made to find the reasons for the genesis and development of UC, its etiology still remains unclear to date.^{4, 5} Current medications used for the UC therapy mainly include some anti-inflammatory drugs, immunomodulators and certain biological agents.⁶⁻⁸ Despite a large number of successful therapeutic outcomes, the mentioned medications have been found to cause various types of side effects in clinical use, and to have low long-term therapeutic efficacy.⁹

Curcumin (CUR) is a hydrophobic polyphenol derivative usually extracted from natural herbal.¹⁰ It has been recognized as a promising anti-inflammatory drug for the UC therapy since it can inhibit the inflammatory pathways, scavenge free radicals and prevent mucosal damage.¹¹ Recently, several studies based on animal models and human clinical trials have demonstrated that CUR has a definite therapeutic efficacy against UC.¹²⁻¹⁴ Importantly, CUR is a relatively safe drug for clinical usage, and it can be orally administered at a large dose as high as 12 g per day for an adult over 3 months.¹⁵ Owing to its high safety, CUR has already been considered as a “generally regarded as safe” compound by FDA.¹⁶ Nevertheless, in many cases, the satisfactory pharmaceutical efficacy of CUR is hard to achieve even at high dosage because CUR is highly hydrophobic with poor pharmacokinetic behaviour, and in addition, it can be fast degraded under physiological conditions.^{17, 18} Some carriers, such as microparticles (MPs), microsponges and nanoparticles (NPs), have thus been deliberately developed and employed as vehicles to protect CUR from degradation or elimination *in vivo* while delivering it toward the targeting tissues.^{12, 19, 20}

Nowadays, orally administrated polymeric NPs is emerging as promising drug carriers for the UC treatment because these specially designed NPs are able to improve the solubility of hydrophobic drugs, prevent the encapsulated drugs from degradation resulted from harsh environment in gastrointestinal tract and increase drug adsorption at the colitis site *via* epithelial permeation and retention effect.²¹ It is now clear that this specific effect reflects a number of factors, including (1) increasingly producing mucus for entrapping the orally delivered NPs on the inflammatory site; (2) generating low-viscosity pores within the mucus layer for facilitating NPs to diffuse through; (3) disrupting colonic epithelial barrier to allow the NPs in intestinal lumen to penetrate into the colitis tissue and (4) accumulation of inflammatory cells that are liable to uptake NPs and retaining them at the inflamed sites.^{3, 22} Based on these successive actions, drug-loaded NPs would be able to penetrate into colitis tissues, resulting in the increasing local drug concentration, the enhanced drug efficacy and the reduced side effect.¹³ In addition, the preference of oral drug administration also provides convenience of self-medication while avoiding pain and contamination *via* parenteral drug administration.²³

In comparison to non-porous polymeric particles, porous polymeric particles have been found to show some advantages such as high drug loading capacity and tailorable drug release rates.²⁴ Accordingly, they have now been commonly used as drug carriers for mucosal disease therapies.²⁵⁻²⁷ However, to the best of our knowledge, no attempt has been made to treat UC by using CUR-loaded porous polymeric NPs so far. Ammonium bicarbonate (ABC), a kind of effervescent porogens, can decompose into gas molecules (namely, ammonia and carbon dioxide) above a certain temperature.²⁸ ABC has thus been used to produce highly-porous polymeric particles due to its gasifiable property.²⁵⁻²⁷ Poly(lactic acid/glycolic acid) (PLGA) is an biodegradable and biocompatible copolymer with well-defined *in vivo* safety and it can be processed into different types of NPs for efficiently delivering hydrophobic drugs.^{21,29}

In the present study, two kinds of NPs were prepared. One is non-porous CUR-loaded PLGA NPs (termed as non-porous NPs) and the other is CUR-loaded porous PLGA NPs that were prepared using ABC as porogen (termed as porous NPs). We further characterized their physicochemical properties (*e.g.* hydrodynamic particle size, zeta potential, morphology and drug loading, as well as encapsulation efficiency). In addition, we comparatively investigated their anti-inflammation properties *in vitro* and therapeutic efficacy against UC *in vivo*.

2. Materials and Methods

2.1. Materials

PLGA (Mw = 38–54 kg/mol), poly(vinyl alcohol) (PVA, 86–89% hydrolyzed, low molecular weight), CUR, dichloromethane (DCM), ABC, Triton X-100 and lipopolysaccharide (LPS) were supplied by Sigma-Aldrich (St. Louis, USA). Paraformaldehyde solution (16%) was purchased from Electron Microscopy Science (Hatfield, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Invitrogen (Eugene, USA). Myeloperoxidase (MPO) Kit and Reactive Oxygen Species (ROS) Kit were supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Dextran sulfate sodium

(DSS, 36–50 kDa) was purchased from MP Biomedicals (Aurora, USA). Buffered formalin (10%) was received from EMD Millipore (Billerica, USA). Hematoxylin and eosin were supplied by Richard-Allan Scientific (Kalamazoo, USA). All commercial products were used without further purification.

2.2. Fabrication of NPs

Porous NPs were prepared by a modified water-in-oil-in-water (W/O/W) double-emulsion solvent evaporation technique, using PVA as an emulsifier and ABC as a porogen. In brief, PLGA (100 mg) and CUR (6 mg) were dissolved in DCM to form an oil phase. 150 μ L of aqueous ABC solution (3%) was then added to the oil phase while homogenizing at a prescribed rate using a Benchmark BV1000 Vortex Mixer. Subsequently, the mixture was sonicated four times (10s for each) at 40% amplitude in an S-450D ice bath (Branson, USA) to form into a primary emulsion. After that, the primary emulsion was added to a 5% PVA solution formed a double emulsion *via* sonication. The so-produced double emulsion was immediately poured into a 0.5% PVA solution. After removing the organic solvent *via* rotary evaporation under reduced pressure, the porous NP suspension was subjected to low-speed centrifugation at 4000 g for 5 min, which gathered larger porous NPs into a pellet while leaving the smaller porous NPs free in the supernatant. Porous NPs in the supernatant were collected *via* high-speed centrifugation at 13000 g for 15 min, followed by two washes. To prevent the aggregation of NPs during lyophilisation, a dilute trehalose solution was added to the final aqueous suspensions. The dry porous NPs were stored at -20 °C for further use. Non-porous NPs were also produced following the same protocol applied to the porous NPs, only without the addition of ABC solution, and used as a control.

2.3. Characterization of NPs

Particle sizes (nm), size distribution and zeta-potential (mV) of non-porous NPs and porous NPs were measured *via* a dynamic light scattering (DLS) technique using a 90 Plus/BI-MAS (Multi-angle particle sizing) instrument or an electric field applied DLS zeta potential analyzer (ZetaPlus, Brookhaven Instruments Corporation). The average values and standard deviations for the diameters of NPs as well as their zeta-potential were calculated using 3 runs.

Surface morphologies of non-porous NPs and porous NPs were viewed using a scanning electron microscope (SEM, JSM-6510LV, JEOL, Japan). A drop of suspension containing non-porous NPs or porous NPs was placed onto a cleaned glass slide and dried before SEM test.

The pore size distributions (PSDs) of porous NPs and non-porous NPs were tested by nitrogen sorption isotherms at 77 K in liquid nitrogen with ASAP-2010 gas adsorption surface analyzer (Delta Analytical Instruments Inc., USA).

CUR load and encapsulation efficiency of non-porous NPs and porous NPs were determined by measuring the intrinsic fluorescence intensity of CUR. Briefly, NPs were dissolved in dimethyl sulfoxide (DMSO), and the CUR fluorescence intensity was tested at 528 nm emission wavelength (λ_{ex} =485nm) using a fluorescence spectrophotometer (RF-5301 PC,

Shimadzu, Japan). Encapsulation efficiency of NPs was calculated as the percentage of actual drug loading amount to the theoretical drug loading amount.

XRD spectra of pure CUR, non-porous NPs and porous NPs were recorded on a XRD-7000 instrument (Shimadzu, Japan) scanning at a speed of 2°/min from 10° to 50° (2θ) under conditions of 40 kV and 30 mA.

2.4. CUR Release profiles from NPs

The CUR release profiles from non-porous NPs and porous NPs were examined using a dialysis method. Briefly, NPs were suspended in PBS (equal to 107 µg CUR), and these suspensions were transferred into dialysis bags (molecular weight cut-off: 10000 Da). Subsequently, these bags were closed tightly at both ends, introduced into centrifuge tubes that were filled with PBS (pH 6.8) containing 1% Tween-80. Here, a small amount of Tween-80 was used to make CUR soluble in the aqueous phase. The centrifuge tubes were then placed in a water bath shaking at 150 rpm at 37 °C. At appropriate time points, a given volume of release medium was withdrawn while replenishing with an equal volume of fresh buffer. The released amount of CUR was measured using the same method mentioned in the cases of measurements for the CUR load and loading efficiency.

2.5. Intracellular uptake of NPs

Raw 264.7 macrophages were seeded in 8-well culture plates (BD Falcon, Bedford, MA, USA) at a density of 3×10^5 cells/well. After being cultured in complete culture medium overnight, cells were treated with serum-free media containing non-porous NPs or porous NPs (CUR equivalent: 50 µM in both cases). Cells without NP treatment were used as a negative control. After co-incubation for 3 h, cells were rinsed with PBS for 3 times to remove the excess of NPs, and then, fixed in 4% paraformaldehyde solution for 15 min. These cells were stained with Hoechst 33352 for imaging with the aid of a confocal laser scanning microscope (Zeiss-800, Germany).

2.6. Flow cytometry (FCM)

Raw 264.7 macrophages were seeded in 12-well culture plates at a density of 1×10^6 cells/well and incubated in complete culture medium overnight. Cells were then exposed to serum-free media containing non-porous NPs or porous NPs, and the CUR concentration in the media was set as 8 µM. Cells without NP treatment were used as a negative control. After co-incubation for various periods of time, cells were thoroughly rinsed with PBS to eliminate excess of NPs. Cells were then collected using cell scraper, and transferred to round-bottom polystyrene test tubes containing FCM buffer. Cell examination was performed on a flow cytometer (ACEA NovoCyte™, USA) using the FITC channel, and a total of 10000 ungated cells was used for analysis.

2.7. MTT assay

Raw 264.7 macrophages and CT-26 cells were seeded in 96-well plates at different densities of 2×10^4 and 8×10^3 cells/well, respectively. After incubation overnight, cells were co-incubated with non-porous NPs or porous NPs (concentrations of CUR, 0-10 µg/mL) for 24 h. At the end of the incubation period, cells were thoroughly rinsed with PBS for 3 times,

and then, incubated with MTT agent (0.5 mg/mL) at 37 °C for 4 h. Thereafter, the media were discarded and DMSO was added to each well prior to spectrophotometric measurements at 570 nm. Untreated cells were used as a negative control, whereas cells treated with 0.5% Triton X-100 were used as a positive control.

2.8. In vitro anti-inflammation properties

Raw 264.7 macrophages were seeded in 48-well plates at a density of 1×10^5 cells/well. After incubation overnight, the medium was exchanged to serum-free medium containing various amounts of non-porous NPs or porous NPs (equivalent CUR concentrations: 2.5 µg/mL, 5 µg/mL and 10 µg/mL, respectively). Cells treated with LPS were used as positive control and cells without LPS treatment were assigned to negative control. After 6 h of co-incubation, the media containing NPs were discarded and complete media were added. After further incubation for 18 h, cells were stimulated with LPS (500 ng/mL) for 3 h. Finally, the concentrations of the main pro-inflammatory cytokines (TNF- α , IL-6 and IL-12) in the supernatants were measured with their cytokine ELISA kits (Biyuntian, Beijing, China), and the ROS productions were also assayed.

2.9. In vivo assessment

In vivo studies were performed on Kunming female mice (8-week old; Chongqing Tengxin Biotechnologies Company, Chongqing, R. R. China). Mice were housed under standard conditions and supplied with food and distilled water. All animal care and studies were approved by Southwest University Institutional Animal Care and Use Committee. The UC model in mice was established *via* the administration of DSS-contained drinking water (3.5%, w/v) for 9 consecutive days, and induced colonic inflammation was assessed during the follow-up treatment period. 4 groups of mice, namely, healthy control group, DSS control group, non-porous NP-treated DSS group and porous NP-treated DSS group, were used in the *in vivo* studies. In the non-porous NP-treated DSS group and the porous NP-treated DSS group, the equivalent CUR dose for mice was 50 mg/kg per day, and the treatment was continued for 9 days. Daily changes in body weight of mice were monitored. At the end of 9-day treatment, mice were sacrificed, and colon and spleen were excised for the subsequent measurements. The resected colon tissues were opened longitudinally and rinsed with PBS to remove luminal content. MPO activity was evaluated using a MPO kit. Colon tissues were sectioned into slices (5 µm in thickness) and stained with hematoxylin and eosin (H&E) for histopathological analysis.

2.10. Statistical analysis

Statistical analysis was performed using ANOVA test followed by a Bonferroni post-hoc test (GraphPad Prism) or Student's *t*-test. Data were expressed as mean $\pm$ standard error of mean (S.E.M.). Statistical significance was represented by **P*<0.05 and ***P*<0.01.

3. Results and discussion

3.1. Fabrication of NPs

As reported, emulsion solvent evaporation technique is a well-established method for fabricating NPs,³⁰ it has thus been employed for preparing non-porous NPs and porous NPs

in the present study. As described in the experimental section, to prepare a primary W/O emulsion, the inner aqueous phase (containing ABC as a gas-foaming agent) was added to a DCM organic phase (containing PLGA and CUR) and the mixture was sonicated in an ice bath to prevent ABC from gasification. Subsequently, we added the primary emulsion dropwise to the outer aqueous phase (containing an emulsifier, PVA), and performed sonication on the mixture to form a W/O/W emulsion based on the Marangoni effect and the capillary break-up process.³¹ During the preparation of NPs *via* this double emulsion technique, many pores would be generated inside NPs because the entrapped ABC will be decomposed into gas molecules (ammonia and carbon dioxide) due to the locally generated heat resulted from the sonication processing. Upon evaporation of organic solvent under reduced pressure, CUR molecules will be concomitantly transferred to the hydrophobic NP domains following the “like dissolves like” principle.³² In the fabrication process of NPs, PVA was used as an emulsifier in considering that it can stably exist in the interface between the oil phase and the water phase, preventing the aggregation of NPs.³³ In addition, a proper amount of PVA can also decrease the binding and activation of lipase/colipase on the surface of NPs,³⁴ slowing the degradation of NPs during their retention in gastric and small intestinal passage.

3.2. Characterization of NPs

Representative SEM images show that the non-porous NPs (Fig. 1a) and porous NPs (Fig. 1b) are spherical in shape. The porous NPs should show porous morphology, but unfortunately, the assumed pores in these porous NPs cannot be visualized using SEM because of the resolution limitation of SEM instrument. To make indirect comparisons based on SEM images, two types of larger PLGA particles, *i.e.*, non-porous MPs and porous MPs, were prepared using ABC as porogen (3%) and following the method mentioned in the experimental section and also described in the literature,²⁴ and representative SEM images for these MPs are presented in Fig. 1c and Fig. 1d. These non-porous MPs and porous MPs were very similar to non-porous NPs and porous NPs in order, except for the differences in the sizes corresponding to MPs and NPs, respectively. It can be seen that non-porous MPs have very smooth surface while porous MPs show a highly porous feature. Images in Fig. 1c and Fig. 1d indirectly suggest that porous NPs have been successfully prepared due to the similarity between MPs and NPs.

Fig. 2a shows the PSDs results of porous NPs and non-porous NPs. It was obvious that porous NPs had a uniform PSD with an average pore size of around 4.0 nm. As expected, no PSD signal was detected for non-porous NPs, which was in good agreement with Fig. 1c.

Particle size, size distribution and zeta potential of NPs play an important role in regulating their drug release, cellular uptake and *in vivo* bio-distributions.³¹ Thus, it is necessary to fabricate NPs with approximately similar parameters. Several main parameters for presently prepared NPs were measured in phosphate buffer with pH of 6.8 since this pH value is nearly the same as that in the colonic lumen, and relevant results are listed in Table 1. Data in Table 1 reveal that the hydrodynamic diameters of non-porous NPs and porous NPs were around 260 nm with a narrow size distribution in term of their very low PDI, and in addition, the two kinds of NPs had negatively charged surface. As supposed, drug loading and

encapsulation efficiency of porous NPs were significantly higher than that of non-porous NPs, which is in good agreement with that indicated in the literature.²⁴ The reason for higher encapsulation efficiency of porous NPs might be ascribed to that the decomposition products of ABC decreased the vapour pressure of organic solvent, and accelerated the solidification of polymeric matrix, resulting in higher encapsulation efficiency.

To further investigate the crystalline/amorphous nature of CUR in NPs, we examined the corresponding XRD patterns of pure CUR, non-porous NPs and porous NPs. As shown in Fig. 2b, the representative XRD diffractogram of pure CUR had a large number of sharp and intense peaks, indicating its highly crystalline nature. In comparison with pure CUR, non-porous NPs and porous NPs exhibited a full absence of the characteristic peaks, suggesting the formation of an amorphous complex between CUR molecules and their matrix.

Therefore, our results clearly demonstrated that crystalline CUR molecules were already converted into amorphous ones inside the carrier materials, which is consistent with that mentioned elsewhere.³⁵

3.3. In vitro release of CUR

Controlled drug release from NPs is a critical prerequisite for drug delivery systems. Fig. 3 shows time-dependent *in vitro* CUR release profiles of NPs. It can be observed that both kinds of NPs exhibited a biphasic CUR release behaviour, characterized by an initial slightly burst release and a followed slower release phase. It has been reported that the drug release from polyester-based NPs is usually correlated to a complex process involving in swelling, erosion and diffusion.³⁶ In the present instance, the initial burst release in both cases can be attributed to the fast diffusion of CUR molecules that were located in superficial layer of NPs. It can also be seen that the CUR release rate of porous NPs was much higher than that of non-porous NPs, and these results are in agreement with those in relation to other types of drug-loaded non-porous MPs and porous MPs.^{24,27,37} At the end of 96-h release, porous NPs sustained released around 76% of initial CUR load, and the cumulative CUR amount released from non-porous NPs was about 58%. The notably higher cumulative CUR amount for porous NPs can be ascribed to a fact that the loaded CUR molecules are able to diffuse more easily through the prebuilt pores existed inside the porous NPs in comparison to non-porous NPs.

3.4. In vitro cytotoxicity

Cytotoxicity is an important issue for drug delivery carriers that are intended to be used for UC therapy. Raw 264.7 macrophages and CT-26 cells were used to test the cytotoxicity of NPs, and data for the cell viability determined using a MTT assay are depicted in ESI Fig. 1. The bar-graphs in ESI Fig.1a show that both non-porous NPs and porous NPs were nearly nontoxic to Raw 264.7 macrophages within the designated dose interval. On the other hand, porous NPs exhibited slight cytotoxicity against CT-26 cells, as shown in ESI Fig.1b. It might be ascribed to the fast CUR release rate of porous NPs (Fig. 3), resulting in the higher intracellular concentration of free CUR in porous NP-treated cells compared with that of non-porous NP-treated cells.

3.5. Intracellular uptake

Efficient intracellular uptake is a major requirement for the therapeutic application of CUR.³⁸ We used confocal microscope to confirm the cellular uptake profiles of non-porous NPs and porous NPs in Raw 264.7 macrophages at the incubation time point of 3 h, as shown in Fig. 4. As expected, the control cells without treatment of NPs showed no fluorescence, whereas clear CUR accumulation was detected from cells treated with non-porous or porous NPs. It was noted that the CUR fluorescence signals in the cells treated with non-porous NPs were much stronger than that of cells treated with porous NPs. These results reveal that non-porous NPs are able to deliver much more CUR into Raw 264.7 macrophages than porous NPs.

To quantitatively compare the cellular uptake efficiency between non-porous NPs and porous NPs, we treated Raw 264.7 macrophages with two kinds of NPs (CUR, 8 μ M), and further examined their cellular uptake efficiencies at various time points (1/3, 2/3, 1, 2, 3 and 5 h). Typical flow cytometric histograms for Raw 264.7 macrophages treated with NPs for 1 h are presented in Fig. 5a. Based on more measurements, quantitative fluorescence intensities for Raw 264.7 macrophages are represented in Fig. 5b. Within the whole measurement time interval changing from 1/3 h to 5h, non-porous NPs already resulted in significantly higher fluorescence intensities in Raw 264.7 macrophages than porous NPs, which was consistent with the results in Fig. 4. The reason might be that non-porous NPs had higher density than porous NPs. Thus, the concentration of non-porous NPs on the cell surface was higher than the initial bulk concentration due to the sedimentation, leading to the increased cellular internalization *in vitro*. As to *in vivo* studies, sedimentation of orally administered NPs should be avoided because of the intestine peristalsis. In addition, we found that intracellular accumulation for both kinds of NPs showed significant time-dependent features when the incubation time was less than 2 h, and after that, no clear differences in the accumulation of NPs were registered for each kind of NPs.

3.6. In vitro anti-inflammation test

To evaluate the *in vitro* anti-inflammation capacities of non-porous NPs and porous NPs, we investigated whether they could block the secretion of the main pro-inflammatory cytokines and ROS production in LPS-stimulated Raw 264.7 macrophages. As shown in Fig. 6a-6c, LPS treatment (positive control) remarkably increased the concentration of pro-inflammatory cytokines (TNF- α , IL-6 and IL-12) in the supernatant, when comparing with that in the negative control. In contrast to this observation, much less secretions of pro-inflammatory cytokines were detected in the supernatant of Raw 264.7 macrophages treated with NPs before LPS stimulation. Notably, the concentrations of pro-inflammatory cytokines were higher in the supernatant of cells treated with non-porous NPs than that matching with porous NPs, even though non-porous NPs were able to transfer much more CUR into the macrophages compared with porous NPs (see Figs. 4 and 5). A possible reason is that porous NPs have higher CUR release rates, inducing higher intracellular CUR concentration than that resulted from non-porous NPs. Furthermore, we found that ROS concentrations in the different treatment groups exhibited a similar pattern (Fig. 6d).

3.7. In vivo therapeutic activity

It has been realized that the DSS-induced mouse model of colitis exhibits histological characteristics similar to that of human UC, including body weight loss, reduction in colon length and disruption of the colonic epithelium layer as well as the infiltration of inflammatory cells.^{39,40} This model was thus used in our *in vivo* studies to evaluate the therapeutic efficacy of NPs.

It can be seen from Fig. 7a that body weight of mice in healthy control group slightly increased over time. On the contrary, mice in the DSS control group had decreased body weight by around 20% after 9-day DSS treatment. Among all the DSS-involved groups, porous NP-treated DSS group showed the smallest body weight loss (approximately 2.1%), which was much lower than that of non-porous NP-treated DSS group. MPO, a hydrogen peroxide oxidoreductase, is usually produced by granulocytic leukocytes, and its activity can be used as an important inflammatory indicator for UC disease. Accordingly, in the present study, MPO activities for colon tissues were measured and results are presented in Fig. 7b. It can be noted that the MPO activity in DSS control group or in non-porous NP-treated DSS group was remarkably higher than that in healthy control group, and in addition, the increase in the MPO activity was strictly correlated with body weight loss of mice when Fig. 7a and Fig. 7b were compared to each other. It is worth noting that there was no significant difference in the MPO activity between the healthy control group and the porous NP-treated DSS group.

As indicated in Fig. 7c, mice in the healthy control group had a spleen weight/body weight ratio of 0.29%. A significant increase in this ratio (up to 0.55%) was observed in the DSS control group. In the porous NP-treated DSS group, only a slight increase in spleen weight/body weight ratio was observed, which was close to that recorded in the healthy control group without significant difference. In particular, mice in the non-porous NP-treated group also had a high spleen weight/body weight ratio of about 0.4% in comparison to that in healthy control group. Additionally, the variation of colon length for the DSS-involved groups as shown in Fig. 7d, exhibited a reverse trend as compared to that of spleen weight/body weight ratio (see Fig. 7c).

Finally, the beneficial effect of porous NPs on DSS-induced UC was confirmed by H&E staining of colon tissue sections. As displayed in Fig. 8a, the image of colon tissue for the mice in the healthy control group exhibited a normal histological structure with no sign of inflammation, which is consistent with that shown in our previous report.⁴¹ The image of colon tissue related to the DSS control group showed clear signs of inflammation, including infiltration of inflammatory cells, depletion of goblet cells and disintegration of colonic epithelia barrier (Fig. 8b). As for the non-porous NP-treated DSS group, the matched image displayed an obvious decrease in the inflammation level (Fig. 8c). In regard to the porous NP-treated DSS group, the image indicated that only a minor damage occurred to the colonic mucosal areas, and nearly no infiltration of inflammatory cells appeared in the mucosa, and in particular, the morphology of colonic tissue for the mice in this group was very similar to that in the healthy control tissue (Fig. 8d).

4. Conclusions

In the present study, non-porous nanoparticles (NPs) and porous NPs were fabricated and used as curcumin carriers for UC therapy. These NPs had desirable diameters (around 260 nm), narrow size distributions (less than 0.07) and slightly negative zeta potentials (about -15 mV). *In vitro* experiments indicated that non-porous NPs and porous NPs were efficiently internalized into macrophages, decreasing the expression levels of pro-inflammatory cytokines (TNF- α , IL-6 and IL-12) and ROS production. Interestingly, we found that oral administration of porous NPs exhibited much better therapeutic efficacy against UC compared with non-porous NPs, indicating that porous NPs was a promising oral drug delivery system for UC therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Representative SEM images and the corresponding size distributions of (a) Non-porous NPs and (b) porous NPs. SEM images of (c) Non-porous MPs and (d) porous MPs for visualization of their surface morphology.

Fig. 2.

(a) Pore size distributions of non-porous NPs and porous NPs; (b) X-ray diffraction patterns of (i) free curcumin, (ii) non-porous NPs and (iii) porous NPs.

Fig. 3.

In vitro release profiles of CUR from non-porous NPs and porous NPs in phosphate buffer (pH 6.8) containing 1% Tween-80 at 37 °C (n=3).

Fig. 4.

Fluorescent images of Raw 264.7 macrophages incubated with non-porous NPs or porous NPs for 1 h (CUR concentration: 50 μ M; scale bar = 20 μ m).

Fig. 5.

Quantitative cellular uptake efficiency of non-porous NPs and porous NPs in Raw 264.7 macrophages. (a) Flow cytometric histograms of fluorescence intensity for cells treated with non-porous NPs and porous NPs (CUR concentration: 8 μ M) for 1 h. (b) Percentage of CUR fluorescence-positive cells after treatment with non-porous NPs and porous NPs (CUR concentration: 8 μ M) for different time points. Each point represents the mean $\pm$ S.E.M. (n=3).

Fig. 6.

In vitro anti-inflammation activity of non-porous NPs and porous NPs against Raw 264.7 macrophages. The secretion of various pro-inflammatory cytokines including (a) TNF- α , (b) IL-6 and (c) IL-12, and the production of (d) ROS. Data are presented as means $\pm$ S.E.M. (n = 5).

Fig. 7. Time-dependent variations of body weight (a), colonic MPO activity (b), spleen weight (c) and colon length (d) for mice treated with non-porous NPs or porous NPs *via* oral administration (data in (b), (c) and (d) were collected from mice having 9-day treatment). Each point represents the mean $\pm$ S.E.M. ($n = 5$). Statistical significance was assessed using the ANOVA test followed by a Bonferroni post-test (* $P < 0.05$; ** $P < 0.01$; ns, not significant).

Fig. 8. Representative H&E staining images of colon tissues obtained from different mice in four groups. (a) Healthy control group, (b) DSS control group, (c) non-porous NP-treated DSS group and (d) porous NP-treated DSS group (Scale bar = 200 μ m).

Table 1
Characteristics of non-porous NPs and porous NPs (mean $\pm$ S.E.M., n=3)

Nanoparticles	Particle size (nm)	PDI	Zeta-potential (mV)	Drug loading (%)	Encapsulation efficiency (%)
Non-porous NPs	264.2 $\pm$ 1.8	0.07	-12.3 $\pm$ 0.7	5.2 $\pm$ 0.3	53.2 $\pm$ 3.3
Porous NPs	260.0 $\pm$ 6.5	0.06	-18.8 $\pm$ 1.3	6.1 $\pm$ 0.2	67.5 $\pm$ 2.0