Facile fabrication of bowl-shaped microparticles for oral curcumin delivery to ulcerative colitis tissue

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A B S T R A C T

Oral microparticles (MPs) have been considered as promising drug carriers in the treatment of ulcerative colitis (UC). Here, a facile strategy based on a conventional emulsion solvent evaporation technique was used to fabricate bowl-shaped MPs (BMPs), and these MPs loaded with anti-inflammatory drug (curcumin, CUR) during the fabrication process. The physicochemical properties of the resultant BMPs were characterized by dynamic light scattering, scanning electron microscope, confocal laser scanning microscope and X-ray diffraction as well as contact angle goniometer. Results indicated that BMPs had a desirable hydrodynamic diameter (1.84 ± 0.20 μm), a negative zeta potential (−26.5 ± 1.13 mV), smooth surface morphology, high CUR encapsulation efficiency and controlled drug release profile. It was found that CUR molecules were dispersed in an amorphous state within the polymeric matrixes. In addition, BMPs showed excellent hydrophilicity due to the presence of Pluronic F127 and poly(vinyl alcohol) on their surface. More importantly, orally administered BMPs could efficiently alleviate UC based on a dextran sulfate sodium-induced mouse model. These results collectively suggest that BMP can be exploited as a readily scalable oral drug delivery system for UC therapy.

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1. Introduction

Ulcerative colitis (UC) is a chronic, relapsing and debilitating inflammatory disease, which mainly distributes erratically in distal bowel [1,2]. It affects millions of individuals worldwide and persists in their lifetime because there is no permanent cure [3]. Therefore, patients with UC have to take the medication throughout their whole life. Currently, the medical treatment of UC mainly relies on the application of aminosalicylates, corticosteroids, immunosuppressive drugs and antibiotics with the goals of controlling inflammation and achieving mucosal healing [4,5]. However, long-term utilization of these medications are associated with serious adverse effects, such as osteoporosis, acute pancreatitis and infection as well as diarrhea [6]. Thus, it is of critical importance to develop alternative agents with high therapeutic efficacy and low side effects.

Curcumin (CUR), a natural dietary substance obtained from turmeric, has attracted increasing attention in UC treatment because it can scavenge free radicals, reduce inflammatory cytokine production and inhibit tumor growth [7,8]. Recent studies have demonstrated that CUR is able to protect mice from UC and maintains remission of UC in patients on a standard therapy [7,9–12]. It is worth noting that no systemic toxicity has been detected with the treatment of CUR. In spite of numerous advantages of CUR, its further application in clinics has been seriously restricted due to its strong hydrophobicity, instability after light exposure, high intestinal metabolic rate and rapid excretion from the body [13–15]. To overcome these problems, a lot of carriers (e.g., pellets, nanoparticles and micelles) have been exploited to deliver CUR to colitis tissue, which could improve the solubility of CUR, protect it from degradation and facilitate it delivery to targeted sites [5,16,17].

Orally administered microparticulate carrier has been recognized as a promising drug delivery system for UC therapy, which benefits from its high drug loading amount, sustained drug release capacity, and colitis tissue-targeted ability based on the epithelial enhanced permeation and retention (eEPR) effect [18]. This effect is mainly attributed to the reduction of mucus thickness, alter-
ation in mucus compositions, and disruption or even complete loss of colonic epithelial layer [19]. The dysfunction of epithelial barrier would facilitate the movement of microparticle (MP) from colonic lumen toward mucosal surface, gradually penetrating into colitis tissue [2]. Therefore, MPs have a good potential to maximize the therapeutic efficacy and minimize the adverse effects. It is known that the shape of carriers is a critical parameter that determines their potential medical applications [20]. Accordingly, modulation of particle shape has become an important strategy in the development of novel therapeutic agents [21–23]. Particularly, bowl-shaped particle is an appealing drug carrier due to their special encapsulation capability and controlled drug release behavior [24]. In previous researches, bowl-shaped particles were prepared with a big opening on their surface [24,25]. In a typical fabrication process, amorphous or semicrystalline polymeric beads were suspended in aqueous solution and swollen after the addition of organic solvents, followed by freezing with liquid nitrogen and evaporation of organic solvents below 0 °C. However, this method required extreme conditions (e.g., liquid nitrogen) and non-FDA-approved polymers, and also affected by multiple critical factors. More recently, mesoporous organosilica particles with bowl-shaped structures were produced, which was also based on very complicated preparation processes [26].

Herein, we described the first attempt to facilely fabricate bowl-shaped CUR-loaded MPs (BMPs) via a well-established double-emulsion solvent evaporation method under mild circumstances. Furthermore, we characterized their physicochemical properties and therapeutic efficacy against an experimental mice model of UC.

2. Materials and methods

2.1. Materials

Poly(lactic acid/glycolic acid) (PLGA, Mw = 38–54 kg/mol), poly(vinyl alcohol) (PVA, 86–89% hydrolyzed, low molecular weight), Pluronic F127 (PF127), CUR, ammonium bicarbonate (ABC), dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were supplied by Sigma-Aldrich (St. Louis, USA). Myeloperoxidase (MPO) Kit was supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, P.R. China). Dextran sulfate sodium (DSS, 36–50 kDa) was purchased from MP Biomedicals (Aurora, USA). Hematoxylin and eosin were supplied by Beyotime Institute of Biotechnology (Shanghai, P.R. China). All commercial products were used without further purification.

2.2. Fabrication of bowl-shaped microparticles (BMPs)

BMPs were fabricated via a water-in-oil-in-water (W/O/W) double-emulsion solvent evaporation method. Briefly, 100 mg of PLGA/PF127 mixture with a weight ratio of 3:1 was dissolved in DCM to form an oil phase. Subsequently, 150 μL of aqueous ABC solution (3%, w/v) was introduced dropwise into the oil phase while homogenizing using a Benchmark BV1000 Vortex Mixer. The mixture was then added into 4 mL of PVA solution (5%, w/v) to form a double emulsion, and this emulsion was poured into diluted 100 mL of PVA solution (0.5%, w/v). After evaporating the organic solvent (DCM) under low pressure for 3 h, BMPs were retrieved by centrifugation at 5000 g for 20 min, followed by 3 washes using ultrapure water. Finally, the collected BMPs were freeze-dried in the presence of trehalose as a cryoprotectant. The dry BMPs were stored at −20 °C for further application.

2.3. Physicochemical characterization of BMPs

Particle sizes (μm) and zeta-potentials (mV) of BMPs were measured using a dynamic light scattering (DLS) technique using a Malvern Zetasizer Nano S90 (Malvern Instruments, London, UK). The average values and standard deviations for the particle sizes of BMPs as well as their zeta-potentials were calculated using 3 runs. The average values were based on the measurements on repeated BMPs.

Surface morphology of BMPs was observed using a scanning electron microscope (SEM, JSM–6510LV, JEOL, Japan). A drop of BMP suspension was placed onto a silicon chip and dried overnight in fume hood. The dried BMPs were pre-processed by coating platinum under vacuum before SEM examination.

Fluorescence microscopic images of BMPs were acquired using a confocal laser scanning microscope (Zeiss–800, Germany) while FITC and DIC channels were used. A drop of BMP suspension was deposited on a cover slide, and dried overnight in fume hood before test.

X-ray diffraction (XRD) spectra of pure CUR, pure PF127 and BMPs were recorded using a Cu Ka-ray at 40 kV and 30 mA ranging from 10° to 50° in an XRD–7000 instrument (Shimadzu, Japan).

2.4. Drug loading and encapsulation efficiency

Loading amount and encapsulation efficiency of CUR in BMPs were studied by measuring the intrinsic fluorescence intensity of CUR. BMPs were dissolved in DMSO, and CUR fluorescence

![Fig. 1. Schematic illustration of the formation process of BMPs based on a double-emulsion solvent evaporation method.](image-url)

<table>
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<th>Table 1</th>
<th>Parameters of BMPs (mean ± S.E.M.; n = 3).</th>
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<td>Particle size (μm)</td>
<td>Zeta potential (mV)</td>
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<td>BMPs</td>
<td>1.9 ± 0.2</td>
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intensity was examined at 425 nm excitation wavelength and 530 nm emission wavelength using a fluorescence spectrophotometer (RF-5301 PC, Shimadzu, Japan). Encapsulation efficiency of BMPs was calculated as the percentage of the actual drug loading amount to the theoretical drug loading amount.

2.5. Wettability measurement

The wettability of BMPs was measured by contact angle relaxation of water droplet using a contact angle goniometer (KRUSS, DSA II GmbH, Germany). BMPs (10 mg) were placed on a clean glass plate, and further planished for contact angle measurements. A drop of deionized water was deposited onto BMP surface, and the contact angle was measured. The droplet images were obtained using a high speed digital camera, and the contact angles were determined accordingly.

2.6. In vitro drug release

In vitro CUR release assays were carried out using a dialysis method, and we used phosphate-buffered saline (PBS, pH = 7.4) and NaAc-HAc buffer (pH = 6.2) as the releasing media. BMP suspension (equal to 250 μg of CUR) was introduced into a regenerated cellulose dialysis bag (molecular weight cut-off = 10000 Da), and the closed bag was put into a centrifuge tube with 20 mL of the releasing medium shaking at 150 rpm and 37 °C. At specific time intervals, outer solution was withdrawn for measurement and replaced by the fresh releasing medium. Finally, the CUR amount in the outer solution was determined using a multifunctional microplate reader (λ<sub>ex</sub>: 425 nm and λ<sub>em</sub>: 530 nm). All of the operations were carried out in triplicates.

2.7. In vivo anti-inflammation properties

Kunming female mice (8 weeks old, Chongqing Tengxin Biotechnologies Company, Chongqing, P. R. China) were housed under standard conditions and supplied with food and distilled water. All animal care and studies were approved by the Southwest University Institutional Animal Care and Use Committee. Mice were treated with DSS-contained drinking water (3.5%, w/v) to establish UC mouse model. Mice were divided into 3 groups, namely healthy control group, DSS control group and BMP-treated DSS group. Mice in the BMP-treated DSS group were orally administered with BMPs every day (5 mg CUR/kg mouse weight). At the end of treatment, mice were sacrificed, and their colons and spleens were excised for the subsequent measurements. The resected colon tissues were opened longitudinally and rinsed with PBS to remove the luminal content. MPO activities were determined using a MPO kit. Colon tissues were sectioned into slices (5 μm) and stained with hematoxylin and eosin (H&E) for histopathological analysis.

2.8. In vivo drug distribution

Kunming female mice (8 weeks old) were used to study the CUR distribution in UC mice. After treatment with DSS solution (3.5%, w/v) for 9 days, all mice were orally administered BMPs at a same dose of 5 mg CUR/kg mouse weight. After 8 or 24 h of administration, mice were sacrificed, and the organs (heart, liver, spleen, lung, kidney, stomach, small intestine and colon) were collected and further stored at −20 °C. Plasma was separated through centrifugation of the whole blood at 5000g for 10 min at 4 °C. All tissues were homogenized in DMSO to extract CUR, and the supernatants were obtained through centrifugation at 10000g for 10 min at 4 °C. The CUR concentrations in the supernatants were examined...
emulsion was formed attributed to the Marangoni effect and the capillary break-up process. In the next step, the entrapped ABC was gradually decomposed into gas (ammonia and carbon dioxide) in a water bath at 37 °C. Since polymers generally had poor thermal conductivities, a temperature gradient was usually formed along the radial direction. Thus, the solidification of W/O/W emulsion started from its surface, which was under reduced pressure and relatively high temperature. The solidified shell had strong mechanical strength to compress gas into the core of particles. When the expansive force of the accumulated gas was bigger than the compressive force of the particle shell, it led to the migration of gas from the interior of particle toward the surface, resulting in the formation of a big void in the core. In the last step, CUR was transferred to the hydrophobic domains of particles with the evaporation of DCM. At the same time, the hydrophobic side chains of emulsifiers (PF127 and PVA) would turn inward and insert into PLGA polymeric matrix while their hydrophilic segments would stretch outward. As a result, the hydrophilic molecular chains presented at the interface of particles separated the oil phase and water phase, preventing the aggregation of particles due to steric hindrance.

3.2. Physicochemical characterizations of BMPs

Particle size and zeta potential are important factors for MPs that directly influence their distribution and penetration in colitis tissues [5,27]. As summarized in Table 1, DLS measurements revealed that the average hydrodynamic particle size of BMPs was approximately 1.9 μm, and their zeta potential was about −26.5 mV. The BMPs exhibited a smooth surface with a bowl shape (Fig. 2a). We also found that they had a narrow size distribution (Fig. 2b). The average particle size of BMPs decreased to 0.87 μm as shown in the SEM images. The difference in the particle sizes determined by SEM and DLS was attributed to the specific surface states of BMPs under the different test conditions, as also found in our previous studies [27]. BMPs were in a swollen state during DLS test, whereas they were fully dehydrated for SEM examination. It was worth noting that their bowl-shaped structure was well maintained after 3 centrifugation cycles, indicating that these BMPs had strong mechanical stabilities for intestinal drug delivery application even with a thin polymeric shell and a big cavity.

Fig. 2c shows a fluorescence image acquired using a confocal laser scanning microscope. It can be clearly seen that CUR was encapsulated in BMPs. Importantly, the bowl shape of BMPs could
be easily observed in the form of a dark core with a green fluorescent corona.

To investigate the interactions between CUR molecules and their polymeric matrixes, we studied the corresponding XRD patterns of pure CUR, pure PF127 and BMPs. As seen in Fig. 3, the representative XRD diffractograms of pure CUR and pure PF127 showed numerous sharp and intense peaks at various 2θ scattering angles, reflecting their highly crystalline nature. On the contrary, the X-ray pattern of BMPs exhibited a complete absence of these peaks, which might be due to the formation of an amorphous state through intermolec-

![Fig. 5](image1.jpg)

**Fig. 5.** Therapeutic effects of orally administered BMPs on DSS-induced colitis in mice. (a) Mouse body weight changes over time, normalized as a percentage of the day-0 body weight and given as the mean for each mice group. (b) Colonic MPO activity, (c) spleen weight and (d) colon length in different mice groups. The MPO results are expressed as units of MPO activity per gram of tissue. Each point represents the mean ± S.E.M. (n=5). Statistical analysis was performed using Student’s t-test (*P<0.05; **P<0.01; ns, not significant).

![Fig. 6](image2.jpg)

**Fig. 6.** Representative H&E-stained colon sections from the (a) healthy control group, (b) DSS control group and (c) BMP-treated group. Scale bar = 200μm.
ular interactions between CUR molecules and polymeric matrices. These results suggest that CUR molecules are molecularly dispersed within the polymeric substrates, which was in a good agreement with a previously published report [28].

As shown in Fig. 4a, the water contact angle of raw PLGA powder was 117.5°. Interestingly, the deposited water droplet on the BMP-coated glass was almost instantly absorbed, and the corresponding water contact angle decreased to 8°, indicating the presence of hydrophilic segments of PF127 and PVA on the surface of BMPs.

3.3. In vitro CUR release behavior

Controlled drug release from particles is an important prerequisite for UC therapy. In vitro release profiles of CUR loading in BMPs as a function of time are shown in Fig. 4b. Compared to the CUR release behavior in PBS, the CUR release rate from BMPs was much slower in NaAc/HAc buffer, which was attributed to the different degradation rates of the carrier matrices in buffers with different pH values. We also found that the CUR release behavior showed an initial rapid release followed by a relatively slow release phase. As reported previously, drug release profiles of polymeric particles were influenced by numerous factors, including matrix swelling, drug diffusion, matrix erosion and polymer degradation [29]. It was speculated that the initial burst release might be due to the diffusion of CUR molecules from the surface layer of BMPs into the releasing solution, while the subsequent CUR migration from the internal to the surface of BMPs led to a moderate and sustained release profile.

3.4. In vivo therapeutic activity

The histological characteristics of the DSS-induced colitis in mice is similar to that of human patient with UC, including a dramatic body weight loss, reduction in colon length, destruction of colonic epithelium and infiltration of inflammatory cells [30]. In addition, this mouse model has a variety of merits, including simplicity, reproducibility and persistence of symptoms with the treatment of DSS [31,32]. Therefore, this mouse model was utilized to evaluate the therapeutic efficiency of orally administered BMPs.

As observed in Fig. 5a, body weight in the healthy control group slightly increased throughout the study, whereas mice in the DSS control group showed an obvious decrease in body weight to 20.1% after 11 days of DSS treatment. However, BMP-treated DSS group exhibited a slight increase in body weight. The observed changes in body weight were correlated with substantial alterations in MPO activities (Fig. 5b), which were related to the concentration of a hydroperoxide oxidoreductase mainly secreted by granulocytic leukocytes. Colonic tissues from the DSS control mice had the highest MPO activity, and that from the healthy control mice showed the lowest MPO activity. Notably, there was no significant difference in MPO activity between the healthy control group and the BMP-treated DSS group.

As shown in Fig. 5c, the DSS control group had a spleen weight of 0.30 g. Interestingly, significantly lower spleen weight (to 0.14 g) was observed in the healthy control group. In the context of BMP-treated DSS group, there was a slight increase in spleen weight to approximately 0.18 g, which was very close to that of the healthy control group. Moreover, colon lengths in different mouse groups exhibited a similar pattern (Fig. 5d).

The therapeutic efficacy of BMPs against DSS-induced UC was further confirmed by histological examinations of colon tissues. As indicated in Fig. 6a, colon tissue from the healthy control group had normal colon histology, and no sign of inflammation or disruption of colon tissue morphology was observed. Consistent with a prior study [33], colon tissues from the DSS control group exhibited clear symptoms of inflammation, including infiltration of inflammatory cells, depletion of goblet cells and disruption of colonic epithelial barrier (Fig. 6b). However, tissues from the BMP-treated DSS group showed no obvious inflammation, and their tissue morphology was much more comparable to that of the healthy colon tissue without obvious symptoms of inflammation (Fig. 6c).

3.5. In vivo drug distribution

The distribution of CUR in internal organs was studied at different time points (8 and 24 h) after oral administration, as shown in Fig. 7. Fig. 7a revealed that CUR dispersed throughout gastrointestinal tract (GIT), and colon had the lowest CUR concentration compared with different sections of GIT after 8 h of administration. In contrast, CUR concentration in colon was increased to 4.8 μg/g tissue at the time point of 24 h, indicating that CUR had a time-dependent accumulation in colitis tissue due to the eEPR effect. Interestingly, it was found from Fig. 7b that CUR was also widely distributed in the five main organs (heart, liver, spleen, lung and kidney) and plasma. These results demonstrate that CUR can be absorbed through GIT and enter the blood stream, and further exert its therapeutic effects against UC in the aspects of a tissue level (colitis tissue) and a systemic level.

4. Conclusions

In the present study, bowl-shaped microparticles (BMPs) were facilely fabricated based on an emulsion-solvent evaporation technique, and further used in the treatment of ulcerative colitis (UC).
The resultant BMPs had a mean hydrodynamic particle size of about 1.9 μm, and their zeta-potential was around –26.5 mV. Their bowl-shaped structure was confirmed using scanning electron microscopy and confocal laser scanning microscopy. Furthermore, we found that CUR could be molecularly dispersed within the polymeric matrix of BMPs. Animal experiments demonstrated that BMPs exhibited an excellent therapeutic efficacy against UC. Collectively, our findings provide clear evidence that BMP could work as an effective formulation for UC therapy.

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