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Colon-targeted dexamethasone microcrystals with pH-sensitive chitosan/alginate/Eudragit S multilayers for the treatment of inflammatory bowel disease

Murtada A. Oshi[a], Muhammad Naeem[a], Junhwan Bae[a], Jihyun Kim[a,b], Juho Lee[a], Nurhasni Hasan[a], Wooseong Kim[a], Eunok Im[a], Yunjin Jung[a], Jin-Wook Yoo[a,*]

[a] College of Pharmacy, Pusan National University, Busandaehak-ro 63 beon-gil, Geumjeong-gu, Busan, South Korea
[b] College of Nanoscience & Nanotechnology, Pusan National University, Busandaehak-ro 63 beon-gil, Geumjeong-gu, Busan, South Korea

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ABSTRACT

Oral colon-targeted drug delivery has gained popularity as an effective strategy for treatment of inflammatory bowel disease (IBD). In this study, we prepared colon-targeted dexamethasone microcrystals (DXMCs) coated with multilayers of chitosan oligosaccharide (CH), alginate (AG), and finally Eudragit S 100 (ES) ($ES_1AG_4CH_5$-DXMCs) using a layer-by-layer (LBL) coating technique. Particle size, surface charge, *in vitro* drug release, and *in vivo* anti-inflammatory activity of $ES_1AG_4CH_5$-DXMCs were evaluated. $ES_1AG_4CH_5$-DXMCs had an average particle size of 2.34 ± 0.19 μm and a negative surface charge of - 48 ± 9 mV. $ES_1AG_4CH_5$-DXMCs demonstrated pH-dependent dexamethasone release, avoiding initial burst drug release in acidic pH conditions of the stomach and small intestine, and providing subsequent sustained drug release in the colonic pH. Importantly, $ES_1AG_4CH_5$-DXMCs exhibited a significant therapeutic activity in a mouse model of colitis compared to other DXMCs. Overall, the LBL-coated DXMCs presented here could be a promising colon-targeted therapy for IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a broad term for chronic inflammatory disorders of the gastrointestinal tract (GIT), which are rapidly growing in both prevalence and incidence across the world (Molodecky et al., 2012). Ulcerative colitis (UC) and Crohn's disease (CD) are the two major types of IBD. In UC, inflammation is limited to the colonic and rectal mucosa and submucosa, while CD is characterized by transmural inflammation that can affect any segment of the GIT (Khor, Gardet, & Xavier, 2011). The etiology of IBD is not yet fully elucidated; however, inappropriate activation of the mucosal immune system is postulated to play a central role (Ardizzone & Bianchi Porro, 2005). The clinical presentation of the disease involves abdominal pain, bloody stools, general fatigue, weight loss, and complete bowel obstruction in severe cases (Baumgart & Sandborn, 2007). Moreover, long-lived patients with IBD were reported to have an increased risk of developing colorectal cancer (Kim & Chang, 2014). There is no cure for IBD, therefore, current treatment options focus on alleviating inflammation and minimizing disease symptoms (Rutgeerts, Vermeire, & Van Assche, 2007).

Dexamethasone is a potent anti-inflammatory corticosteroid that has been used to treat mild to moderate forms of IBD (Sood, Midha, Sood, & Awasthi, 2002). However, long-term use of corticosteroids is often associated with serious systemic side effects such as hypertension, hyperglycemia, and immune suppression (Buchman, 2001; Mahadevan, 2004). Moreover, there are increasing reports of corticosteroid dependence and resistance associated with their systemic administration in patients with IBD (Munkholm, Langholz, Davidsen, & Binder, 1994). To avoid the systemic side effects and improve the local bioavailability of corticosteroids at target inflamed tissues, a wide range of oral colon-targeted drug delivery systems, such as pH-sensitive polymer coating systems and enzyme-dependent release systems, have been investigated (Kesisoglou & Zimmermann, 2005). Particularly, micro and nanoparticle-based systems employing the aforementioned approaches have been widely studied with the aim of improving the targeting ability to inflamed colonic tissues and reducing the side effects of the conventional dosage forms (Naeem, Kim, Cao, Jung, & Yoo, 2014; Watts et al., 1992). However, dexamethasone-loaded particulate systems have yet to be successful due to their poor drug loading efficiency, which is mainly caused by external phase crystallization during encapsulation (Cavalli, Trotta, & Tumiatti, 2006; Gómez-Gaete, Tsapis, Besnard, Bochot, & Fattal, 2007).

* Corresponding author at: College of Pharmacy, Pusan National University, Busan 609-735, South Korea.
E-mail address: jinwook@pusan.ac.kr (J.-W. Yoo).

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In this study, we prepared pure dexamethasone microcrystals (DXMCs) with pH-sensitive layer-by-layer (LBL) coating for colon-targeted drug delivery. Layer-by-layer coating is a powerful technique for surface modifications, by which oppositely charged polyelectrolytes can be alternately coated on the surfaces of several materials including drug crystals (Ai, Jones, de Villiers, & Lamb, 2003). In this study, the LBL coating technique was employed to avoid early drug release from DXMCs in the upper GIT (stomach and small intestine) before reaching the colon, thereby reducing systemic side effects and increasing the drug availability in the colon (Mohtashamian & Boddohi, 2017). We hypothesized that LBL coating of DXMCs with chitosan oligosaccharide (CH), alginate (AG) (CH/AG) multilayers and an outermost pH-sensitive Eudragit® S 100 (ES) layer could lead to colon-targeted drug release and thus, improve the therapeutic efficacy of the drug. Chitosan is a natural cationic polysaccharide that has long been used in colon-targeted drug delivery (Chourasia & Jain, 2004). In this study, we used CH instead of high molecular weight chitosan, which dissolves in pH 7.4 and thus enables an electrostatic interaction with both AG and ES during LBL-coating process of DXMCs. AG is a biodegradable and biocompatible anionic polysaccharide that also has wide applications in oral colon-targeted drug delivery (Chourasia & Jain, 2004). ES, an anionic copolymer based on methacryslic acid and methacrylate, is a pH-sensitive polymer insoluble at pH values below 7; therefore, it is widely used as a coating material in oral colon-targeted drug delivery systems (Naeem et al., 2018). Alternate coating of DXMCs with multilayers of the polyelectrolytes was confirmed by zeta potential measurement and the shapes of the coated DXMCs were observed by scanning electron microscopy (SEM). Drug release was investigated in different pH conditions resembling those of the GIT. Finally, the therapeutic activity of LBL-coated DXMCs (LBL-DXMCs) was evaluated in a mouse model of dextran sulfate sodium (DSS)-induced colitis.

2. Materials and methods

Source for some methods are shown in the Supplementary data.

2.1. Materials

Dexamethasone was purchased from Wako (Osaka, Japan). Chitosan oligosaccharide (Mw 4000–6000 with deacetalytion degree of 89.69%) was purchased from Kitto life (Seoul, South Korea). Sodium alginate (Mw 80,000–120,000 with mannuronate/guluronate ratio of 1.56) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Eudragit® S 100 (Mw ~125,000) was generously gifted by Evonik Korea Ltd (Seoul, Korea). Dextran sulfate sodium (Mw 36,000–50,000) was obtained from MP Biomedicals (Irvine, CA, USA). Mouse interleukin 6 (IL-6) DuoSet® ELISA kit was purchased from R&D Systems Inc. (Minneapolis, USA), and mouse tumor necrosis factor alpha (TNF-α) ELISA MAX® kit was purchased from Biolegend Inc. (San Deigo, USA). Primary anti-E-cadherin was purchased from BD Transduction Laboratories (San Jose, USA). Alexa Flour 488-conjugated AffiniPure goat anti-mouse IgG was obtained from Jackson ImmunoResearch Inc. (West Grove, PA, USA). All other chemicals, reagents, and solvents were of the highest commercially available analytical grade.

2.2. Preparation of LBL-DXMCs

Layer-by-layer coating of DXMCs was accomplished by a sonication-assisted LBL coating technique (Santos et al., 2015). Briefly, 75 mg of dexamethasone powder were suspended in 15 mL of CH solution (2 mg/mL in 0.05% NaCl, pH 7.4) and sonicated (90 W/cm^2) for 30 min at 4 °C to fabricate DXMCs coated with CH as a stabilizer (CH_1-DXMCs). Then, CH_1-DXMCs were collected by centrifugation at 6000 × *g* for 10 min, washed thrice with 0.05% NaCl to remove excess CH, and resuspended in an AG solution (2 mg/mL in 0.05% NaCl, pH 7.4). The suspension was gently shaken for 20 min at room temperature to allow AG to coat CH_1-DXMCs. Then, the microcrystals were collected by centrifugation at 6000 × *g* for 10 min and washed thrice with distilled water to remove excess AG. By the end of this step, DXMCs were coated with both CH and AG. Then, the aforementioned coating process (alternating CH and AG layers, followed by the same washing steps) was repeated until the desired number of CH/AG multilayers was coated on the DXMCs. For coating with the outermost ES layer, CH/AG-coated microcrystals were shaken in ES solution (2 mg/mL in 0.05% NaCl, pH 7.4) for 20 min at room temperature and washed as mentioned above. The resultant LBL-DXMCs were lyophilized and stored at 4 °C for subsequent use.

2.3. DSS-induced colitis and drug treatment

All *in vivo* procedures were conducted in compliance with the guidelines for animal studies approved by Pusan National University (Busan, South Korea). Colitis was induced in male ICR mice (30–35 g body weight) by administration of the colitogenic compound DSS at a concentration of 2.5% w/v in drinking water for 7 days. Age-matched mice received normal tap water and served as a healthy control group. Mice were divided into 5 groups: healthy control; colitis control; and colitis-induced mice treated with CH_1-DXMCs, LBL-DXMCs coated with 10 consecutive CH/AG multilayers (AG_5CH_5-DXMCs), and LBL-DXMCs coated with 10 consecutive CH/AG multilayers and an outermost ES layer ($ES_1AG_4CH_5$-DXMCs) (n = 8/group). The 3 treated groups were orally administered an equal dose of dexamethasone (0.1 mg/kg) by gastric gavage starting from day 8 to day 14.

2.4. Evaluation of in vivo anti-inflammatory activity of LBL-DXMCs

2.4.1. Clinical assessment of colitis

Clinical assessment of induced colitis involved measuring the disease activity index (DAI) in a blind fashion from day 1 to day 14 of the study (Cooper, Murthy, Shah, & Sedergran, 1993). The DAI is obtained by adding the scores for body weight, stool consistency, and fecal bleeding. At the end of the study, all mice were sacrificed by CO_2 asphyxiation and colons were collected. Colon lengths were measured, and colon weight/length ratios were calculated as gross macroscopic indicators of colitis severity.

2.4.2. Histological assessment of colitis

For histological assessment of colitis, colon samples approximately 1 cm in length were cut and immediately fixed in 10% formaldehyde. Fixed colon samples were embedded in paraffin, sectioned at 5-μm thickness using a microtome, and stained with hematoxylin and eosin (H&E). An arbitrary histological scoring was used to quantify colitis severity in the colon samples (Cummins et al., 2008). Moreover, colon samples from each mice group were embedded in paraffin and sectioned at 5-μm thickness for immunostaining. Then, they were incubated with the primary antibody anti-E-cadherin at 4 °C overnight and then with the secondary antibody Alexa Flour 488-conjugated goat anti-mouse IgG for 1 h at room temperature. Finally, they were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and analyzed using CLSM.

3. Results and discussion

3.1. Preparation and characterization of LBL-DXMCs

In the current study, LBL-DXMCs were prepared by coating DXMCs with CH/AG multilayers (*e.g.*, AG_5CH_5-DXMCs) or with CH/AG multilayers and an outermost ES layer (*e.g.*, $ES_1AG_4CH_5$-DXMCs) using a sonication-assisted LBL coating technique (Fig. 1). Dexamethasone powders are commercially available as micronized particles; however, they are not suitable for colon-targeted LBL coating due to their irregular and large particle size caused by significant particle aggregation in water as shown in Fig. 1 and 2 A. Thus, to obtain freely dispersed

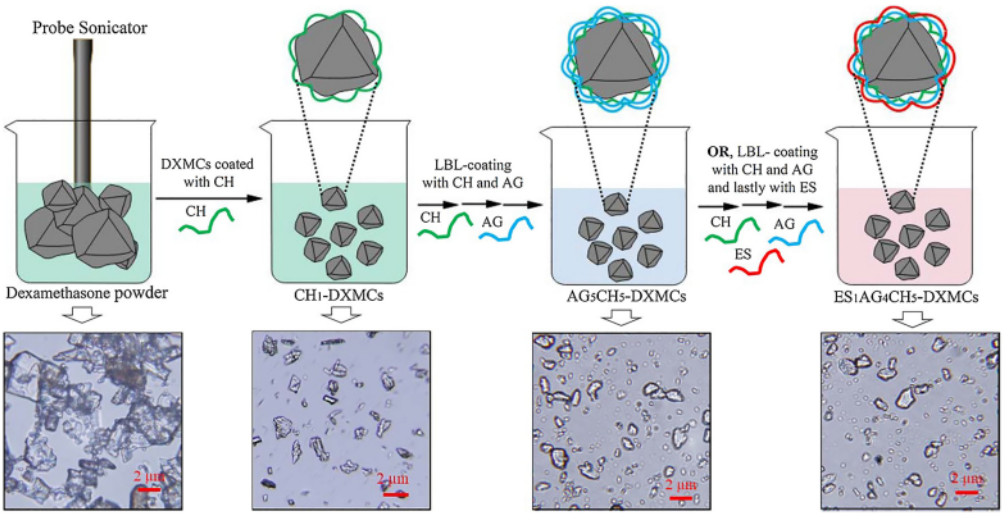

Fig. 1. Schematic diagram of the preparation of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs. DXMCs were coated with alternating layers of CH and AG only or with alternating layers of CH and AG and finally with ES as the outermost layer using a sonication-assisted LBL coating technique.

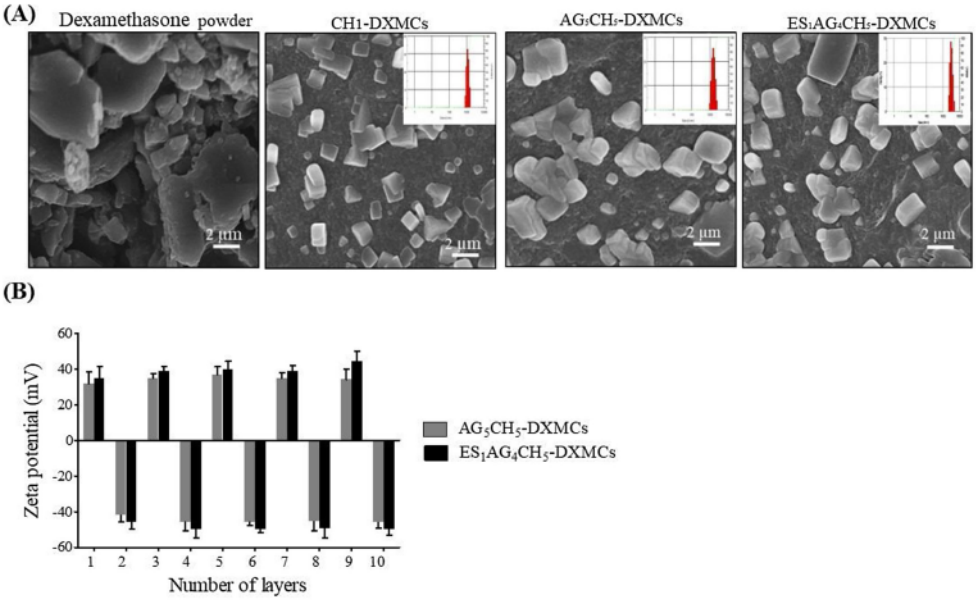

Fig. 2. Characterization of the physicochemical properties of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs. (A) SEM images and particle size distribution of dexamethasone powder, CH_1-DXMCs AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs. (B) Changes in zeta potential of DXMCs during LBL coating with CH, AG, and ES. CH_1-DXMCs, DXMCs coated with CH; AG_5CH_5-DXMCs, DXMCs coated with CH/AG multilayers; $ES_1AG_4CH_5$-DXMCs, DXMCs coated with CH/AG multilayers and an outermost ES layer.

Table 1
Physicochemical characteristics of dexamethasone powder, CH_1-DXMCs, AG_5CH_5-DXMCs, and $ES_1AG_4CH_5$-DXMCs.

Formulation	Particle size (μm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)	Drug content (%)
Dexamethasone powder	n.a.[a]	–	–	–	–
CH_1-DXMCs	1.91 ± 0.11	0.15 ± 0.01	+ 34 ± 6	–	96 ± 3
AG_5CH_5-DXMCs	2.29 ± 0.10	0.29 ± 0.07	−48 ± 9	91 ± 5	90 ± 2
$ES_1AG_4CH_5$-DXMCs	2.34 ± 0.19	0.23 ± 0.08	−48 ± 9	93 ± 6	91 ± 2

All measurements were performed in triplicate, values are expressed as mean ± SD (n = 3).
[a] Particle size could not be measured due to severe aggregation.

DXMCs with a uniform particle size, a suspension of dexamethasone powder in a 2 mg/mL solution of CH was prepared and sonicated at 90 W/cm^2 for 30 min. As shown in Figs. 1 and 2A, the resulting CH_1-DXMCs did not aggregate in the water, demonstrated smaller mean particle size (1.91 µm ± 0.11) than that of dexamethasone powder, and exhibited narrow particle size distribution as indicated by a PDI of 0.15 ± 0.01 (Table 1). We attempted to further decrease the particle size by applying higher ultrasonication power or longer ultrasonication time; however, particle size did not substantially decrease below 1.91 µm. When dexamethasone powder was ultrasonicated in the absence of CH, DXMCs quickly aggregated and precipitated, indicating that CH functions as a stabilizer for DXMCs. The morphologies of dexamethasone powder and CH_1-DXMCs were analyzed by SEM (Fig. 2A). Chitosan-coated DXMCs exhibited a positive zeta potential of + 34 ± 6 mV, which was converted from a negative charge of dexamethasone powder (Pargaonkar, Lvov, Li, Steenekamp, & de Villiers, 2005), indicating the successful coating of CH. Moreover, CH_1-DXMCs demonstrated a high drug content of up to 96%.

Prepared CH_1-DXMCs were further coated with CH/AG multilayers or with CH/AG multilayers and an outermost ES layer to prepare LBL-DXMCs. The mean particle size of LBL-DXMCs was found to range between 2–2.5 µm with a narrow size distribution (Table 1, Supplementary Table S1 and S2). It was previously reported that particles smaller than 10 µm can easily diffuse through the mucus and accumulate selectively in inflamed colon areas (Lamprecht, Schafer, & Lehr, 2001). The morphologies of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs were analyzed by SEM (Fig. 2A). The zeta potential of the DXMCs changed between positive (up to + 45 mV) when coated with CH and negative (up to - 48 mV) when coated with AG or AG and ES (Fig. 2B). This positive/negative charge alternation indicated the successful deposition of each polyelectrolyte layer onto DXMCs. The drug content and encapsulation efficiency of LBL-DXMCs with different numbers of CH/AG multilayers and LBL-DXMCs with different numbers of CH/AG multilayers and an outermost ES layer are shown in Table 1, Supplementary Table S1 and S2. High drug content is one of the notable advantages of LBL-coated drug crystals (Santos et al., 2015).

3.2. Study of pH-dependent drug release from LBL-DXMCs

We assessed the colon-targeting potential of CH_1-DXMCs and LBL-DXMCs by testing the *in vitro* drug release in a medium with a gradually increasing pH from 1.2 to 6.8 to 7.4. These pH values were selected as they resemble the pH values in the human stomach, small intestine, and colon, respectively (Naeem et al., 2018). Chitosan-coated DXMCs showed a burst drug release (> 90%) within ~2-3 h at the stomach and small intestine pH values (1.2 and 6.8) (Fig. 3A). In the management of IBD, an ideal colon-targeted drug delivery system avoids the initial unwanted burst drug release in the stomach and small intestine, and provides a sustained drug release in the colon to ensure maximum drug availability at the inflamed colon areas (Nidhi et al., 2016). In this study, prevention of the initial unwanted burst drug release at pH 1.2 and 6.8 was achieved by coating DXMCs with CH/AG multilayers using a sonication-assisted LBL coating technique. As shown in Fig. 3A, LBL-DXMCs coated with CH/AG multilayers released ~65-85% of dexamethasone in the first 5 h at pH 1.2 and 6.8. However, although the drug release rate from LBL-DXMCs was slowly decreased by increasing the number of CH/AG multilayers, they all still exhibited a burst drug release profile at pH 1.2 and 6.8, and at colon pH (pH 7.4), all LBL-DXMCs continued to rapidly release the drug (Fig. 3A). To further decrease the drug release at pH 1.2 and 6.8, DXMCs were coated with alternating CH/AG multilayers and an outermost ES layer using a sonication-assisted LBL coating technique. ES provides gastric protection to the oral drug delivery system and enables the drug release specifically in the colon (Naeem et al., 2018).

As shown in Fig. 3B, coating LBL-DXMCs with CH/AG multilayers and finally with an outermost ES layer suppressed dexamethasone release in the first 5 h at pH 1.2 and 6.8 compared to LBL-DXMCs coated with CH/AG multilayers without ES. As shown in Fig. 3B, all LBL-DXMCs released approximately 45–60% of dexamethasone in the first 5 h at pH 1.2 and 6.8. It was found that LBL-DXMCs coated with less than 10 layers of CH/AG and ES ($ES_1AG_1CH_2$-DXMCs, $ES_1AG_2CH_3$-DXMCs, and $ES_1AG_3CH_4$-DXMCs) did not reduce the burst drug release in the first 5 h at pH 1.2 and 6.8. However, LBL-DXMCs coated with 10 layers of CH/AG and ES ($ES_1AG_4CH_5$-DXMCs) showed a sudden decrease in the drug release, with less than 20% of dexamethasone released in the first 5 h at pH 1.2 and 6.8. Moreover, by increasing the pH of the medium to 7.4, $ES_1AG_4CH_5$-DXMCs, unlike other LBL-DXMCs, avoided complete drug release and released the remaining dexamethasone (~80%) in a sustained fashion over approximately 15 h (Fig. 3B). The mechanism of the sudden change is not clear yet;

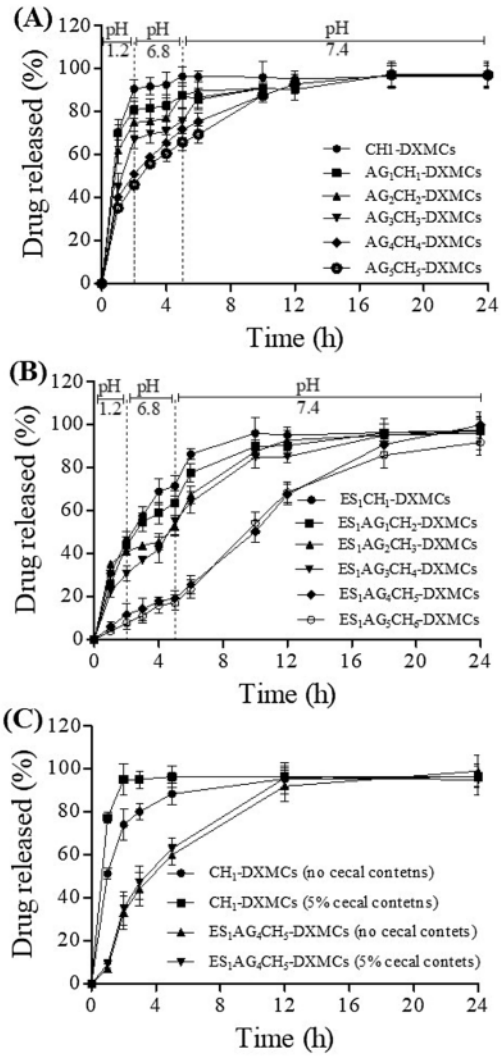

Fig. 3. Study of the pH-dependent drug release of dexamethasone from LBL-DXMCs. (A) Drug release profiles of LBL-DXMCs coated with different numbers of CH/AG multilayers. (B) Drug release profiles of LBL-DXMCs coated with different numbers of CH/AG multilayers and finally an outermost ES layer. (C) *In vitro* drug release from CH_1-DXMCs and $ES_1AG_4CH_5$-DXMCs in the presence and absence of the cecal contents. Results are presented as mean ± S.D (n = 3).

however, we presume that there is a critical number of ES/CH/AG multilayers in the presence of the final ES layer, above which the multilayer structure becomes strong enough to prevent the drug release. This hypothesis was partially supported by the release profile of $ES_1AG_5CH_6$-DXMCs, which exhibited a similar drug release with $ES_1AG_4CH_5$-DXMCs, indicating that the sudden decrease of drug release can be obtained above the critical multilayer number (Fig. 3B). However, further studies are warranted to confirm the hypothesis.

After the drug release studied in gradually increasing pH resembles of human GIT pH, the next inquiry was whether $ES_1AG_4CH_5$-DXMCs can release a drug sufficiently in the diseased colon after oral administration. The drug release from CH_1-DXMCs and $ES_1AG_4CH_5$-DXMCs were studied in the presence and absence the of cecal contents (Fig. 3C). In the case of CH_1-DXMCs, an increased drug release rate was observed in the presence of cecal contents as compared to their absence, indicating the enzymatic degradation of CH layer. In contrast, the drug release rate of $ES_1AG_4CH_5$-DXMCs was not significantly affected by cecal contents. We presume that Eudragit/alginate/chitosan multilayers of LBL-DXMCs forms a rigid complex which may function as a barrier for bacterial activities. Results of the pH-dependent drug release supported our hypothesis that $ES_1AG_4CH_5$-DXMCs decrease the drug release at pH 1.2 and 6.8 for 5 h, followed by the sustained release thereafter at pH 7.4. Therefore, $ES_1AG_4CH_5$-DXMCs, unlike CH_1-DXMCs and LBL-DXMCs coated with CH/AG multilayers, can efficiently minimize dexamethasone loss from the stomach before reaching the colon and thus, increase the drug availability in the colon, which is a vital feature for oral colon-targeted drug delivery.

3.3. The effect of the outermost ES layer on the colon-targeting potential

The main goal of colon-targeted drug delivery systems in management of IBD is to deliver the maximum amount of the drug to the colon. In this study, we investigated the effect of the outermost ES coating on the colon-targeting potential of LBL-DXMCs by evaluating the particle size and shape of $ES_1AG_4CH_5$-DXMCs in a medium with a gradually increasing pH from 1.2 to 6.8 to 7.4, compared to AG_5CH_5-DXMCs (Fig. 4A). As shown in Fig. 4B, the mean particle size of AG_5CH_5-DXMCs was decreased by changing the pH of the medium; its initial mean particle size was 2.29 μm and it was reduced to ~1.73 μm, ~0.88 μm, and ~0.31 μm at pH 1.2, 6.8, and 7.4, respectively. In contrast, the particle size of $ES_1AG_4CH_5$-DXMCs was maintained at pH 1.2 and 6.8 and then was reduced at pH 7.4, at which the ES layer dissolves. The initial mean particle size of $ES_1AG_4CH_5$-DXMCs before starting the experiment was ~2.34 μm and then it became ~2.12 μm and ~2.02 μm at pH 1.2 and 6.8, respectively, and finally was reduced to 1.19 μm at pH 7.4 over 10 h. Moreover, SEM images revealed the pH-dependent changes in $ES_1AG_4CH_5$-DXMCs particle shape at pH 7.4 (Fig. 4C). This was further confirmed by CLSM (Fig. 4C), where the outermost ES layer (green) of the DXMCs (red) is shown to be intact at pH 1.2 and 6.8 for 6 h, while it dissolves leading to size reduction at pH 7.4. Particle shape and size of AG_5CH_5-DXMCs were markedly changed by changing the medium pH from 1.2 to 6.8 to 7.4, while those of $ES_1AG_4CH_5$-DXMCs did not change at pH 1.2 and 6.8, and only demonstrated a notable shape change and size reduction at pH 7.4. These results further support the results of the pH-dependent drug release study.

3.4. Evaluation of the in vivo anti-inflammatory activity of LBL-DXMCs

3.4.1. Clinical assessment of colitis

To study the effect of the outermost ES coating on DXMCs anti-inflammatory activity *in vivo*, CH_1-DXMCs, AG_5CH_5-DXMCs, and $ES_1AG_4CH_5$-DXMCs were administered to mice in a model of DSS-induced colitis and their effects were compared. The DSS model was chosen because the pathohistopathology of DSS-induced colitis resembles that of human colitis (Chassaing, Aitken, Malleshappa, & Vijay-Kumar, 2014). The clinical features of colitis and the effects of CH_1-DXMCs, AG_5CH_5-DXMCs, and $ES_1AG_4CH_5$-DXMCs were characterized by evaluating the DAI results of mice in all the study groups throughout the study period (Fig. 5A). The DAI in the healthy control mice remained stable without changes throughout the study days, while mice in the colitis group demonstrated elevated DAI values, indicating the severity of the induced colitis. Mice treated with CH_1-DXMCs and AG_5CH_5-DXMCs exhibited elevated DAI values by ~14% and ~10%, respectively. In contrast, mice treated with $ES_1AG_4CH_5$-DXMCs demonstrated a significant decrease ($P < 0.001$) in the DAI compared to the colitis control group. In addition to monitoring the DAI, colon lengths were measured, and colon weight/length ratios were calculated at the end of the study (Fig. 5B and C). In the colitis group, colon lengths were shortened, and colon weight/length ratios were elevated, indicating severe inflammation. The colon lengths and colon weight/length ratios of mice treated with CH_1-DXMCs and AG_5CH_5-DXMCs were not improved after the treatment period. However, treatment with $ES_1AG_4CH_5$-DXMCs was associated with a significant increase in the colon length ($P < 0.05$) and a significant decrease in the colon weight/length ratio ($P < 0.01$) compared to the colitis control group.

Results of CH_1-DXMCs and AG_5CH_5-DXMCs treatment agree with their drug release profiles. Both CH_1-DXMCs and AG_5CH_5-DXMCs demonstrated a burst drug release profile at pH 1.2 and 6.8, which resemble the stomach and small intestine pH values. This resulted in a reduction in the colon drug concentration. In this study, the dissolved part of the drug from CH_1-DXMCs and AG_5CH_5-DXMCs failed to improve the clinical features of colitis after systemic absorption. This may be because the used dose of dexamethasone was lower than the dose needed for systemic treatment of IBD. A daily intravenous dose of 100 mg of dexamethasone has been proven as an effective dose for treatment of IBD in a prospective, open-label trial (Sood et al., 2002). In this study, treatment of DSS-induced mice with $ES_1AG_4CH_5$-DXMCs was found to effectively mitigate the clinical symptoms of colitis. This effect may be attributed to the ability of the outermost ES layer of $ES_1AG_4CH_5$-DXMCs to protect the drug in acidic conditions and to release the drug in a sustained profile in the colon (pH 7.4).

3.4.2. Histological assessment of colitis

Histological features of colitis were also investigated in the different study groups. The H&E-stained colon sections of mice from the 5 study groups and the histological score of each group are shown in Fig. 6A. Colons of healthy mice showed intact mucosa without any signs of mucosal or submucosal damage. In contrast, colons from mice in the colitis control group demonstrated mucosal epithelial damage and loss of goblet cells, which indicates the severity of DSS-induced colitis. Histological findings from colons of mice treated with CH_1-DXMCs were similar to those of the colitis control group, while mice treated with AG_5CH_5-DXMCs demonstrated weak improvement in histological features compared to the colitis control group. Interestingly, colons of mice treated with $ES_1AG_4CH_5$-DXMCs demonstrated a remarkable improvement in histological features of colitis compared to the colitis control group. The above findings were confirmed by histological scoring of colon tissue sections from all study groups (Fig. 6A). In addition, immunohistochemical evaluation of colitis severity was achieved by E-cadherin staining of colon sections from all study groups (Fig. 6B). E-cadherin is an important protein in adherens junctions between cells and plays a key role in histopathological features of IBD (Mendoza & Abreu, 2009). Colons of healthy mice demonstrated a high staining intensity of E-cadherin, while colons from the colitis control group demonstrated low E-cadherin intensity, indicating disruption of the tight junctions between the cells by DSS administration. Colon sections of mice treated with CH_1-DXMCs and AG_5CH_5-DXMCs also demonstrated low E-cadherin intensity, similar to the colitis group, while colon sections of mice treated with $ES_1AG_4CH_5$-DXMCs demonstrated elevated levels of E-cadherin compared to the colitis control group (Fig. 6B). Overall, these histological findings further confirmed the accumulation of dexamethasone in the colons of mice treated with

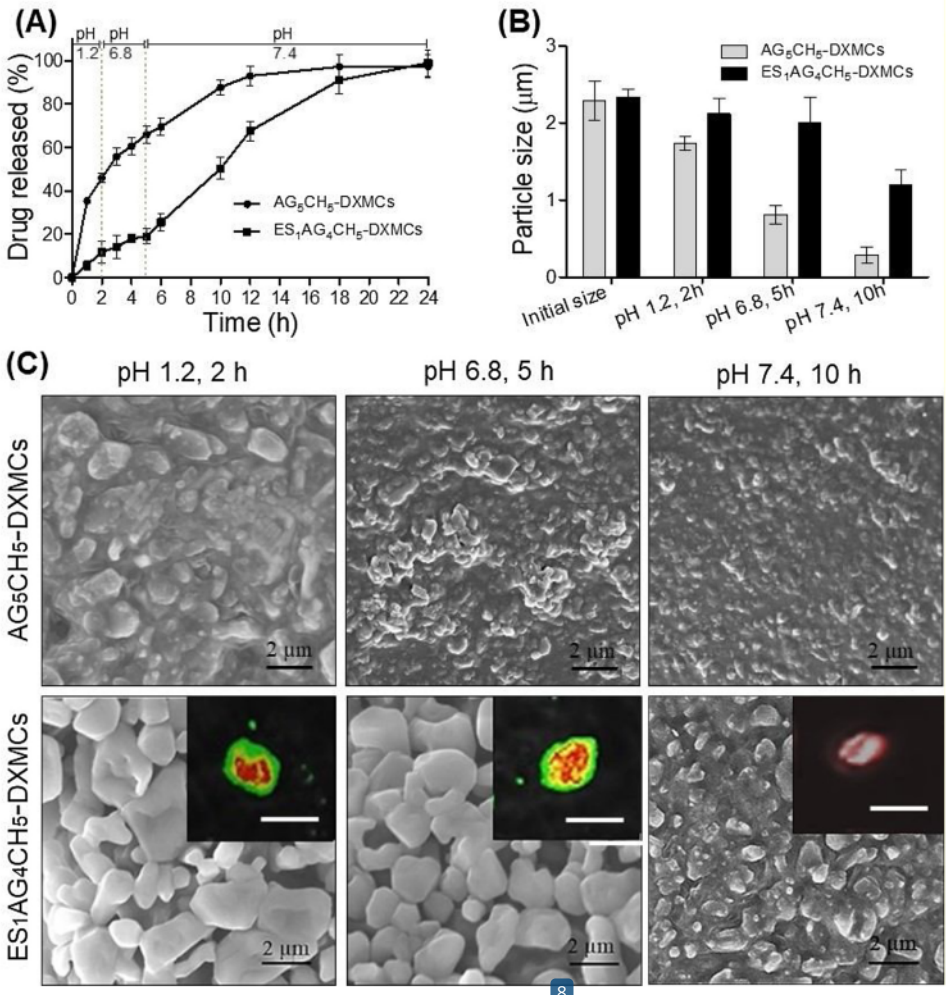

Fig. 4. Characterization of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs particle size and shape in a medium with gradually increasing pH. (A) Drug release profile of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs in a medium with gradually increasing pH. (B) Changes in particle size of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs at different pH values. (C) Series of SEM images showing the changes in shape of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs + series of CLSM images of $ES_1AG_4CH_5$-DXMCs (upper parts of SEM images, scale bar = 2 µm). DXMCs (Red color) and FITC-albumin conjugated (green color). AG_5CH_5-DXMCs, DXMCs coated with CH/AG multilayers; $ES_1AG_4CH_5$- DXMCs, DXMCs coated with CH/AG multilayers and an outermost ES layer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

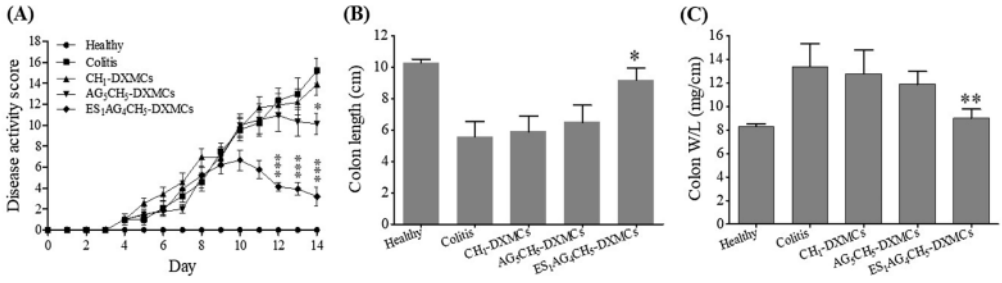

Fig. 5. Clinical assessment of colitis in the different study groups. (A) disease activity index values of mice in the different study groups throughout the study days. (B) colon length. (C) colon weight/length (W/L) ratio of mice in the different study groups. Data are presented as mean ± SD (n = 8/group). *,**,***: $P < 0.05$, 0.01, and 0.001, respectively, *versus* colitis group. CH_1-DXMCs, DXMCs coated with CH; AG_5CH_5-DXMCs, DXMCs coated with CH/AG multilayers; $ES_1AG_4CH_5$-DXMCs, DXMCs coated with CH/AG multilayers and an outermost ES layer.

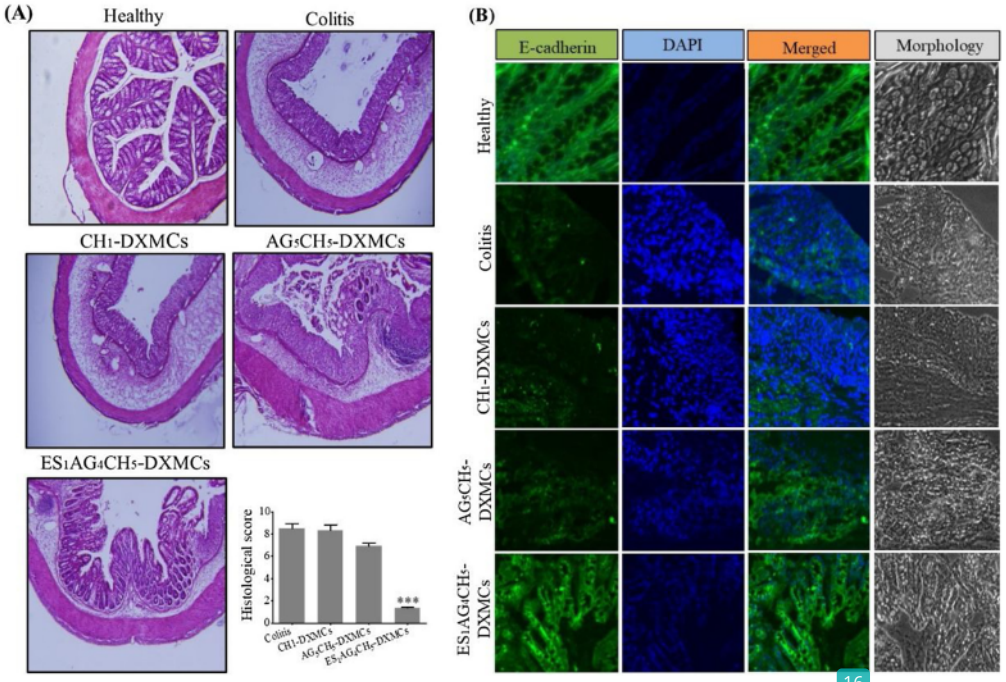

Fig. 6. Histological and immunohistochemical assessment of colitis. (A) Histological assessment of colitis in colon tissues from the different study groups. Data are presented as mean ± SD (n = 8/group). ***: $P < 0.001$ *versus* colitis group. (B) E-cadherin immunostaining images of colon tissue sections from the different study groups. The green color represents E-cadherin and the blue color represents DAPI used to stain cell nuclei. DAPI, 4′,6-diamidino-2-phenylindole.

$ES_1AG_4CH_5$-DXMCs, thereby significantly suppressing inflammation in this group compared to the other treatment groups and to the colitis group.

3.4.3. Determination of MPO and proinflammatory cytokine levels

The therapeutic effects of CH_1-DXMCs, AG_5CH_5-DXMCs, and $ES_1AG_4CH_5$-DXMCs in DSS-induced colitis were further assessed by determining the levels of MPO and the proinflammatory cytokines TNF-α and IL-6 in colon samples. Myeloperoxidase concentrations in colons of mice from different study groups are shown in Fig. 7A. Myeloperoxidase is an important indicator of the degree of neutrophils infiltration and thus, of colitis severity (Wéra, Lancellotti, & Oury, 2016). Tissue MPO levels were found to be within the normal range in colon samples of healthy control mice, while they were elevated in all other groups, which indicates the disease induced by DSS administration. However, tissue MPO levels in mice treated with $ES_1AG_4CH_5$-DXMCs were significantly lower ($P < 0.001$) than in the colitis control group. Concentrations of IL-6 and TNF-α in different study groups are shown in Fig. 7B and C. Since IL-6 and TNF-α play a vital role in inflammatory responses in colitis pathogenesis, blockade of their production is an important approach in the treatment of IBD (Uings et al., 2005). Similar to MPO, IL-6 and TNF-α levels were within the normal range in colon samples of healthy control mice, while they were elevated in colon samples of colitis control mice. Treatment of mice with CH_1-DXMCs and AG_5CH_5-DXMCs did not lower the elevated concentrations of both cytokines. However, treatment with $ES_1AG_4CH_5$-DXMCs significantly lowered TNF-α and IL-6 concentrations ($P < 0.001$) compared to colitis control mice. Collectively, these results further confirm the high accumulation of dexamethasone in the colons of mice treated with $ES_1AG_4CH_5$-DXMCs compared to other treatments.

4. Conclusion

In this study, colon-targeted DXMCs coated with CH/AG multilayers (AG_5CH_5-DXMCs) and with CH/AG multilayers and an outermost ES layer ($ES_1AG_4CH_5$-DXMCs) were successfully fabricated using a

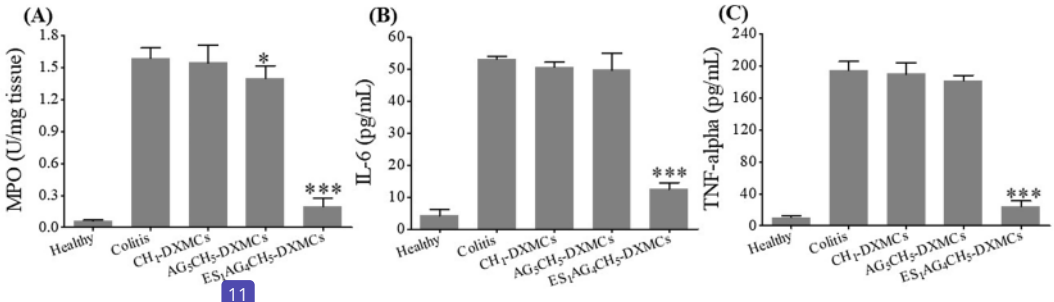

Fig. 7. Average concentrations of MPO, IL-6, and TNF-α in colon samples of mice from the different study groups. (A) Tissue MPO levels, (B) IL-6 levels, (C) TNF-α levels. Data are presented as mean ± SD (n = 8/group). *,***: $P < 0.05$ and 0.001, respectively, *versus* colitis group.

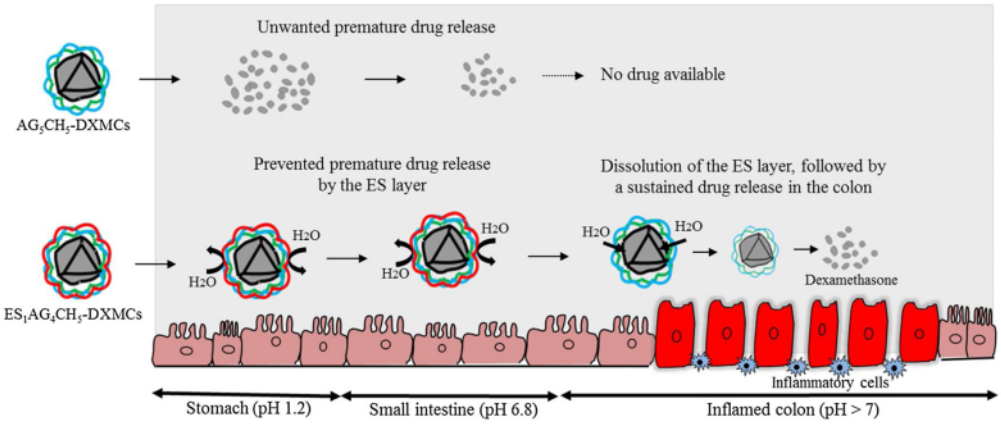

Fig. 8. Proposed mechanism of dexamethasone release from AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs at pH values mimicking different regions of the GIT. AG_5CH_5-DXMCs, DXMCs coated with CH/AG multilayers; $ES_1AG_4CH_5$-DXMCs, DXMCs coated with CH/AG multilayers and an outermost ES layer.

sonication-assisted LBL coating technique. The successful coating of DXMCs with CH, AG, and ES was confirmed by zeta potential measurement after each coating step. Fabricated AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs had average diameters of 2.29 ± 0.1 μm and 2.34 ± 0.19 μm, respectively, with a negative surface charge for both. Study of pH dependent drug release from LBL-DXMCs showed that AG_5CH_5-DXMCs exhibited an undesirable burst drug release profile in media mimicking the stomach and the small intestine, while $ES_1AG_4CH_5$-DXMCs, which is coated by the outermost pH-responsive ES layer, provided significant protection against drug dissolution at pH values of the upper GIT and released the drug in a sustained manner at colonic pH. The proposed drug release mechanisms of AG_5CH_5-DXMCs and $ES_1AG_4CH_5$-DXMCs in different parts of the GIT are summarized in Fig. 8. Furthermore, the pH-responsive and colon-targeted $ES_1AG_4CH_5$-DXMCs markedly improved the therapeutic outcomes in a model of DSS-induced colitis in mice as compared to CH_1-DXMCs and AG_5CH_5-DXMCs. Therefore, our findings demonstrated that pH-sensitive LBL-DXMCs provide a promising colon-targeted delivery system that can be applied in the treatment of IBD.

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Conflict of Interest

There is no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2018.06.107.

References

Ai, H., Jones, S. A., de Villiers, M. M., & Lvov, Y. M. (2003). Nano-encapsulation of furosemide microcrystals for controlled drug release. *Journal of Controlled Release, 86*(1), 59–68.
Ardizzone, S., & Bianchi Porro, G. (2005). Biologic therapy for inflammatory bowel disease. *Drugs, 65*(16), 2253–2286.
Baumgart, D. C., & Sandborn, W. J. (2007). Inflammatory bowel disease: Clinical aspects and established and evolving therapies. *Lancet, 369*(9573), 1641–1657.
Buchman, A. L. (2001). Side effects of corticosteroid therapy. *Journal Of Clinical Gastroenterology, 33*(4), 289–294.
Cavalli, R., Trotta, F., & Tumiatti, W. (2006). Cyclodextrin-based nanosponges for drug delivery. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 56*(1), 209–213.
Chassaing, B., Aitken, J. D., Malleshappa, M., Vijay-Kumar, M., et al. (2014). Dextran sulfate sodium (DSS)-induced colitis in mice. In John E. Coligan (Ed.). *Current protocols in immunology* 104, Unit-15.25.
Chourasia, M. K., & Jain, S. K. (2004). Polysaccharides for colon targeted drug delivery. *Drug Delivery, 11*(2), 129–148.
Cooper, H. S., Murthy, S. N., Shah, R. S., & Sedergran, D. J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Laboratory Investigation, 69*(2), 238–249.
Cummins, E. P., Seeballuck, F., Keely, S. J., Mangan, N. E., Callanan, J. J., Fallon, P. G., ... Taylor, C. T. (2008). The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis. *Gastroenterology, 134*(1), 156–165.
Gómez-Gaete, C., Tsapis, N., Besnard, M., Bochot, A., & Fattal, E. (2007). Encapsulation of dexamethasone into biodegradable polymeric nanoparticles. *International Journal of Pharmaceutics, 331*(2), 153–159.
Kesisoglou, F., & Zimmermann, E. M. (2005). Novel drug delivery strategies for the treatment of inflammatory bowel disease. *Expert Opinion on Drug Delivery, 2*(3), 451–463.
Khor, B., Gardet, A., & Xavier, R. J. (2011). Genetics and pathogenesis of inflammatory bowel disease. *Nature, 474*(7351), 307–317.
Kim, E. R., & Chang, D. K. (2014). Colorectal cancer in inflammatory bowel disease: The risk, pathogenesis, prevention and diagnosis. *World Journal of Gastroenterology : WJG, 20*(29), 9872–9881.
Lamprecht, A., Schafer, U., & Lehr, C. M. (2001). Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. *Pharmaceutical Research, 18*(6), 788–793.
Mahadevan, U. (2004). Medical treatment of ulcerative colitis. *Clin Colon Rectal Surg, 17*(1), 7–19.
Mendoza, J. L., & Abreu, M. T. (2009). Biological markers in inflammatory bowel disease: Practical consideration for clinicians. *Gastroentérologie Clinique Et Biologique, 33*(Supplement 3), S158–S173.
Mohtashamian, S., & Boddohi, S. (2017). Nanostructured polysaccharide-based carriers for antimicrobial peptide delivery. *Journal of Pharmaceutical Investigation, 47*(2), 85–94.
Molodecky, N. A., Soon, I. S., Rabi, D. M., Ghali, W. A., Ferris, M., Chernoff, G., ... Kaplan, G. G. (2012). Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology, 142*(1), 46–54 e42; quiz e30.
Munkholm, P., Langholz, E., Davidsen, M., & Binder, V. (1994). Frequency of glucocorticoid resistance and dependency in Crohn's disease. *Gut, 35*(3), 360–362.
Naeem, M., Kim, W., Cao, J., Jung, Y., & Yoo, J.-W. (2014). Enzyme/pH dual sensitive polymeric nanoparticles for targeted drug delivery to the inflamed colon. *Colloids and Surfaces B: Biointerfaces, 123*, 271–278.
Naeem, M., Oshi, M. A., Kim, J., Lee, J., Cao, J., Nurhasni, H., ... Yoo, J.-W. (2018). pH-triggered surface charge-reversal nanoparticles alleviate experimental murine colitis via selective accumulation in inflamed colon regions. *Nanomedicine: Nanotechnology, Biology and Medicine*.
Nidhi, Rashid, M., Kaur, V., Hallan, S. S., Sharma, S., & Mishra, N. (2016). Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: A brief review. *Saudi Pharmaceutical Journal, 24*(4), 458–472.
Pargaonkar, N., Lvov, Y. M., Li, N., Steenekamp, J. H., & de Villiers, M. M. (2005). Controlled release of dexamethasone from microcapsules produced by polyelectrolyte layer-by-layer nanoassembly. *Pharmaceutical Research, 22*(5), 826–835.
Rutgeerts, P., Vermeire, S., & Van Assche, G. (2007). Mucosal healing in inflammatory bowel disease: Impossible ideal or therapeutic target? *Gut, 56*(4), 453–455.

Santos, A. C., Pattekari, P., Jesus, S., Veiga, F., Lvov, Y., & Ribeiro, A. J. (2015). Sonication-assisted layer-by-layer assembly for low solubility drug nanoformulation. *ACS Applied Materials & Interfaces, 7*(22), 11972–11983.

Sood, A., Midha, V., Sood, N., & Awasthi, G. (2002). A prospective, open-label trial assessing dexamethasone pulse therapy in moderate to severe ulcerative colitis. *Journal Of Clinical Gastroenterology, 35*(4), 328–331.

Uings, I., Puxeddu, I., Temkin, V., Smith, S. J., Fattah, D., Ray, K. P., ... Levi-Schaffer, F. (2005). Effects of dexamethasone on TNF-alpha-induced release of cytokines from purified human blood eosinophils. *Clin Mol Allergy, 3*(1), 5.

Watts, P. J., Barrow, L., Steed, K. P., Wilson, C. G., Spiller, R. C., Melia, C. D., ... Davies, M. C. (1992). The transit rate of different-sized model dosage forms through the human colon and the effects of a lactulose-induced catharsis. *International Journal of Pharmaceutics, 87*(1), 215–221.

Wéra, O., Lancellotti, P., & Oury, C. (2016). The dual role of neutrophils in inflammatory bowel diseases. *Journal of Clinical Medicine, 5*(12), 118.

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