Structural Characteristics of Inulin and Microcrystalline Cellulose and Their Effect on Ameliorating Colitis and Altering Colonic Microbiota in Dextran Sodium Sulfate-Induced Colitic Mice

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ABSTRACT: Several studies have reported that dietary fibers (DFs) from plants may exert beneficial effects on inflammatory bowel disease. In the present study, we investigated the structural differences of soluble DF (inulin) and insoluble DF (microcrystalline cellulose, MCC) and their effects on the intestinal barrier integrity, gut microbiota community, and inflammation response in mice with dextran sodium sulfate (DSS)-induced colitis. Mice were fed for 21 days with diets containing inulin or MCC (2.5 g/kg body weight), and colitis was induced by administration of DSS (4% w/v) in drinking water during the last 8 days of experimentation. The results showed that inulin and MCC differ in morphology and structure. MCC exhibited a smaller particle size, a larger specific surface area, and higher thermal stability than inulin. In addition, both inulin and MCC restored various physical signs (body weight, colon weight and length, disease activity index score, and infiltration of inflammatory cells), gut barrier function (as evidenced by the increased expression of claudin-3, claudin-7, ZO-2, occludin, JAM-2, and MUC-3 and the decreased activity of myeloperoxidase activity), downregulation of mRNA expression of proinflammatory cytokines (caspase-1, NLPR3, TLR4, TNF-α, and IL-1β), and modulation of colon microbiota community. Taken together, the present study demonstrates that DFs differ in morphology and structure and ameliorate DSS-induced colitis in mice by blocking proinflammatory cytokines, reinforcing gut barrier integrity, and modulating gut microbiota. Therefore, DFs, especially inulin, are promising dietary supplements to alleviate intestinal inflammation.

1. INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn’s disease (CD) and ulcerative colitis (UC), affects up to 500 per 10000 people in the Western world. In China, the morbidity of UC shows a year-by-year increasing trend. Classical symptoms of IBD include prolonged periods of abdominal pain, diarrhea, hematochezia, and other gastrointestinal symptoms, and these are usually difficult to cure due to recurrent attacks. Moreover, IBD not only affects the patients’ quality of life but also frequently progresses to colon cancer.

Growing evidence indicates that gut bacteria play an important role in the pathogenesis of IBD and its further development into chronic disease. A decrease in Bacteroidetes and Firmicutes species together with an increase in virulent Escherichia coli (E. coli) species has been found in the intestinal flora of IBD patients. The interaction between enteric microorganisms and their metabolites and the mucosal immune system plays a pivotal part in gut health and in preventing the progression of IBD. The changes in mucosal flora, associated with damage to the intestinal mucosal barrier caused by toxins, initiate pathologic immune responses leading to acute and chronic colitis. Thus, the reduction of gut inflammation may be realized by ameliorating the integrity of the gut mucosal barrier. Due to the undesirable side effects of long-term use of traditional treatments of IBD including antibiotics, aminosalicylates, and glucocorticoids, improved therapeutic strategies for IBD are urgently needed.

Dietary intervention can regulate the diversity of the bacterial flora and the mucosal immune system to improve the intestinal health and overall health of the body and has shown great potential for the anesis or prevention of IBD. Dietary fiber (DF) consists of nondigestible edible carbohydrates and their analogues. While these substances cannot be digested or absorbed in the small intestine, they can be...
fermented in whole or in part by microorganisms in the hindgut. DFs have been reported to have numerous biological activities, including antioxidant, anti-inflammatory, and antitumor, and are commonly classified into insoluble (IDF) and soluble (SDF) dietary fiber according to their solubility in water. The SDFs include inulin, pectin, and guar gum, and part hemicellulose, while IDFs mainly include cellulose, hemicellulose, and lignin. Intake of DFs may have various physiological effects on human health based on different physicochemical properties. Inulin, a β-(2–1) linked fructan polymer, can be found in chicory roots, beet roots, leeks, and other natural sources. In addition, as a prebiotic fructan polymer, inulin is used as functional additives of yogurt, sausages, DF supplements during pregnancy, and so forth and play an important role in the food and health product industry. In animals, dietary inulin and MCC cannot be hydrolyzed by endogenous enzymes, but they are readily fermented by microbes in the colon and gut, leading to the hindgut. The combined soluble fibers, with high viscosity and fermentability, have been found to attenuate systemic inflammation, promote intestinal microbiota homeostasis, and improve the intestinal morphological integrity of mice suffering from obesity induced by a high-fat diet. Though some studies have found an anti-inflammatory action of DFs, it is not clear if DFs can promote anesis or prevention of gut injury.

The objective of the current study was to investigate whether inulin and MCC have an inhibitory effect on gut inflammation. To this end, diarrhea in BALB/c mice caused by the administration of dextran sodium sulfate (DSS) was used as a mouse model of IBD. The effects of inulin and MCC on gut barrier repair and their anti-inflammatory mechanism of action were assessed by qRT-PCR. Furthermore, the microbial community in the colons of mice was analyzed by 16S rRNA sequencing.

2. RESULTS AND DISCUSSION

2.1. Particle Size Distribution of DFs. Particle size, including porosity and surface area, plays an important role in the physicochemical and functional properties of DF. By affecting fermentation and fecal bulking, it can promote the body’s absorption of glucose and cholesterol and intestinal health. The particle size distribution and specific surface area of different DFs are shown in Table 1. The results showed that the d (0.5) and d (0.9) of MCC (59.204 ± 3.840 and 128.284 ± 3.976 μm, respectively) were smaller than those of inulin (95.919 ± 3.215 and 428.326 ± 14.083 μm, respectively), which means inulin presents the larger average particle size. The particle size and specific surface area are usually negatively correlated, so MCC likely has a larger specific surface area than inulin.

| items         | inulin      | MCC         |
|---------------|-------------|-------------|
| mannose       | 1.9         | 1.8         |
| ribose        | ND          | ND          |
| rhamnose      | 0.1         | ND          |
| glucuronic acid| ND          | 0.1         |
| galacturonic acid| 0.3      | 4.0         |
| glucosamine   | 0.5         | 0.1         |
| glucose       | 5.9         | 87.3        |
| galactosamine | 3.6         | ND          |
| galactose     | ND          | 0.6         |
| xylose        | 4.0         | ND          |
| arabinose     | 2.2         | 6.0         |
| fructose      | 81.6        | ND          |

aMCC: microcrystalline cellulose. ND means not determined.

2.2. Monosaccharide Composition. The results (Table 2) of monosaccharide composition analysis showed that the main monosaccharides compositions of inulin were fructose, glucose, xylose, and galactosamine at a ratio of 81.6:5.9:4.3:6, which is similar to the previous report that fructose is the main composition in inulin. The monosaccharide compositions of MCC were glucose, arabinose, galacturonic acid, and mannose at a ratio of 87.3:6.4:0.6, which is similar to the sesame MCC that glucose makes up nearly 90% of total sugar.

Table 1. Mean Particle Diameter and Specific Surface Areas of DFs

| samples   | d (0.1) (μm)     | d (0.5) (μm)     | d (0.9) (μm)     | homogeneity | specific surface area (m²/g) |
|-----------|-----------------|-----------------|-----------------|-------------|-----------------------------|
| inulin    | 21.473 ± 0.658a | 95.919 ± 3.215a | 428.326 ± 14.083a | 1.190 ± 0.556a | 0.404 ± 0.019a              |
| MCC       | 22.558 ± 0.596b | 59.204 ± 3.840b | 128.284 ± 3.976b | 0.561 ± 0.184b | 0.883 ± 0.014b              |

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2.3. SEM Analysis. The morphology of DFs was observed by scanning electron microscopy (SEM). Clear differences in microstructures were observed between inulin and MCC at 2000-fold magnification. The image of inulin showed a spherical shape with a smooth surface (Figure 1A), while MCC displayed folds with a smaller particle size that afforded it a larger specific surface area (Figure 1B). Micropores increased the surface area of MCC, leading to capillary action, which was important for glucose absorption, water retention capacity, oil absorption capacity, and so forth.

2.4. FT-IR Analysis. The chemical structures of the DFs were analyzed by Fourier transform infrared (FT-IR) spectroscopy (Figure 2). The absorption band in the range 3000–3600 cm⁻¹ for inulin and MCC corresponds to hydroxyl groups (–OH) and the band around 2927 cm⁻¹ corresponds to C–H stretching in cellulose, hemicellulose, and lignin. The carbonyl group band at 1647.21 cm⁻¹ corresponds to the characteristic bond vibrations of the ester and carboxyl groups (–COOCH₃ and –COOH) of ferulic and p-coumaric acids in...
hemicellulose. The band at 1028 cm\(^{-1}\) in DFs refers to C–O–C stretching of β-1,4-glycosidic bonds among D-glucose units in cellulose. In addition, the peak strength increased around 1130 and 3354 cm\(^{-1}\), indicating the presence of cellulose, which was more pronounced in the MCC.

### 2.5. XRD Analysis

The X-ray diffraction (XRD) patterns of DFs are shown in Figure 3. There were no sharp peaks found in inulin around 2θ = 22.3 and 34.46\(^\circ\), indicating its amorphous pattern. Such amorphization plays a critical role in the enhanced water solubility of inulin. In contrast, the XRD spectra of MCC displayed several sharp peaks at 2θ = 22.3 and 34.46\(^\circ\), indicating that MCC was semicrystalline. The crystalline structure of MCC will guarantee a natural role and proper stimulation of microorganisms in the colon.

### 2.6. Thermal Analysis

The thermal stability of different DFs is shown in Figure 4. Thermogravimetry (TG) curves showed that thermal degradation of inulin and MCC was a multistage thermal degradation process. The first degradation peaks of inulin and MCC occurred at 55.30 and 97.91 °C and ended at 125.41 and 150 °C, respectively. The main cause of heat loss at this stage may be water loss in DFs. The second thermal degradation occurred in the range of 264.8 and 300 °C, possibly due to the breakdown of glycosidic bonds and the decomposition of carbohydrates. Compared to inulin, the degradation peak of the differential TG (DTG) curves of MCC exhibited polysaccharide characteristics and was exothermic. The fluctuation of the thermal degradation temperature may be caused by the different molecular properties and physical states of DFs. As shown in Figure 4, the thermal characteristics of DFs are relatively complex, and there were differences in the differential scanning calorimetry (DSC) spectra between different DFs. The degradation peak corresponding to water release appeared near 120 °C. Compared with inulin, MCC showed fewer endothermic peaks and exothermic peaks below 250 °C, indicating that MCC has better thermal stability than inulin.

### 2.7. Effects of DFs on Body Weight and Intestinal Histology

Inulin DF possesses high solubility and high fermentability, while MCC exhibits the opposite, that is, low solubility and low fermentability. DSS, a water-soluble polysaccharide sulfate with direct toxic effects on intestinal epithelial cells, can destroy the integrity of intestinal barrier...
resulting in the onset of acute colitis. To directly assess the effect of solubility and fermentability of DFs on the amelioration of DSS-induced colitis in mice, we compared the body weight and intestinal histology of mice that were fed inulin or MCC in their diet. As shown in Figure 5A, mice in the inulin, MCC, and DSS groups suffered significant weight losses (p < 0.05) compared with the control group, and this trend continued until the end of the experiment. However, administration of inulin significantly reduced the weight loss of DSS-induced mice from day 3 to day 7 by 264.32, 136.11, 77.05, 80.32, and 60.25%, respectively. A similar trend was seen for the MCC group, although in this case, the difference with the DSS group was only significant at day 7, reaching 32.93%. Weight changes between the two DF groups were not significantly different.

As shown in Figure 5B, the disease activity index (DAI) clinical scores of the inulin group at day 3 and beyond were lower than those of the DSS group with clinical scores decreasing by 100, 75, 50, 33.33, and 5.26%, respectively. Overall, inulin was better at preventing weight loss than MCC.

Colon length is generally regarded as a morphologic marker for inflammation degree, and the shortening of the colon in mice has been correlated with histologic changes. Oral administration of DSS decreased the length and weight of the colon in the mice of the DSS group, while feeding inulin partially prevented the decrease in the colon length (by 42.99%, p < 0.05) and the decrease in the colon weight (by 33.67%, p < 0.05) compared with the DSS group (Figure 5C–E). The reason may be that DSS induced goblet cell depletion in the distal side of the colon, resulting in the contraction of colon length and loss of colon weight. Furthermore, Figure 5E represents the morphological characteristics of colons. The colon length of DSS-treated mice was significantly shorter than that in mice in normal groups (p < 0.05). Meanwhile, feeding MCC also somewhat alleviated the decrease in colon length.

Figure 5. DFs ameliorate DSS-induced colitis in mice. (A) Bodyweight change, (B) clinical score, (C) colon length, (D) colon weight, (E) morphological characteristics of colons, (F) histological analysis of normal colonic mucosa, (G) histological analysis of colitis group, (H) histological analysis of 2.5 g/kg body weight inulin, and (I) histological analysis of 2.5 g/kg body weight MCC. Con, control mice fed regular diets; DSS, diet included 4% (w/v) DSS during the last 8 days of the experiment; inulin, diet included 2.5 g/kg body weight inulin and 4% (w/v) DSS; MCC, diet included 2.5 g/kg body weight MCC and 4% (w/v) DSS. Different letters above the graph points or columns in panels A–D indicate significant differences (p < 0.05).
intestinal epithelium. MUC3 is expressed in goblet and intestinal mucosal proteins, act as an important component of the intestinal barrier through the adhesion and intercellular junction connection system that is formed by epithelium.45 MUC3 is an important intestinal mucin that can bind many intestinal pathogens and viruses, preventing them from attaching to the surface of intestinal cells.52 The results showed that oral administration of inulin and MCC can increase the expression of MUC3, thus inhibiting enteropathogenic E. coli adherence.49 The overexpression of TJ genes and of the MUC3 protein has been reported to result in an amelioration of DSS-induced colitis,54,55 consistent with our results.

Myeloperoxidase (MPO) is considered a potential marker of tissue inflammation, tissue damage, and neutrophil infiltration.40 DSS-induced colitis is characterized by diffuse inflammation and the accumulation of neutrophils in the colonic mucosa with high expression of MPO.3 The MPO activity in the colons of the DSS group had increased by 317.66 and 121.88% compared with the control group and the inulin group, respectively (p < 0.05) (Figure 6B), indicating that inulin intake reduced the infiltration of colonic mucosa by neutrophils. Another study similarly found that feeding 10% guar gum decreased the level of MPO activity in mice with DSS-induced colitis (p < 0.05) and increased the expression of TJ proteins. On the other hand, the MPO activity in the MCC group did not differ significantly from that in the inulin or the DSS groups. These results indicate that DFs, especially inulin, protected the mice from the DSS-induced increase in intestinal permeability.41 In addition, MUC-3 mRNA expression was higher in the inulin and MCC groups by 456.28 and 474.26%, respectively, than in the DSS group (p < 0.05). MCC3 is an important intestinal mucin that can bind many intestinal pathogens and viruses, preventing them from attaching to the surface of intestinal cells. The results showed that oral administration of inulin and MCC can increase the expression of MUC3, thus inhibiting enteropathogenic E. coli adherence. The overexpression of TJ genes and of the MUC3 protein has been reported to result in an amelioration of DSS-induced colitis, consistent with our results.

2.8. Effect of DFs on Barrier Function. The internal environment of the body is isolated from the external environment, and the integrity of tissues is maintained by the intestinal barrier through the adhesion and intercellular junction connection system that is formed by epithelium.42 Intestinal permeability is regulated by epithelial adherens junctions (JAMs) and tight junctions (TJs, including ZO1, occludin, claudins, etc.), and these can be compromised by enteric dysbacteriosis or other stimuli.43 Increased intestinal permeability can aggravate certain diseases such as IBD and irritable bowel syndrome.44 Furthermore, MUCs, critical intestinal mucosal proteins, act as an important component of the mucus layer and provides an insoluble barrier to protect intestinal epithelium.45 MUC3 is expressed in goblet and columnar cells of the surface epithelium of the colon, is associated with the maturation of intestinal epithelial, and may be necessary to maintain normal epithelial cell function during inflammation.46 In this study, the relative expression of TJ and JAM genes, including claudin-3, claudin-7, ZO-2, occludin, JAM-2, and MUC-3, was found to be lower in colons of the DSS group than in the control group (p < 0.05) (Figure 6A), which means inulin can repair the intestinal barrier function and restore intestinal permeability.41 In addition, MUC-3 mRNA expression was higher in the inulin and MCC groups by 456.28 and 474.26%, respectively, than in the DSS group (p < 0.05). MCC3 is an important intestinal mucin that can bind many intestinal pathogens and viruses, preventing them from attaching to the surface of intestinal cells. The results showed that oral administration of inulin and MCC can increase the expression of MUC3, thus inhibiting enteropathogenic E. coli adherence. The overexpression of TJ genes and of the MUC3 protein has been reported to result in an amelioration of DSS-induced colitis, consistent with our results.

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especially inulin, effectively decreased the production of IL-1β, TLR4, and TNF-α in DSS-treated mice (p < 0.05), which were 47.45, 29.96, and 53.72% lower, respectively, than in the DSS group. Additionally, the addition of MCC also led to a downward trend in the expression of those inflammatory cytokines which failed to reach statistical significance. Depending on its gene activation level, caspase-1 can either cause programmed cell death or promote cell survival, and it can stimulate cell survival response and is beneficial for cell growth when expressed at low levels.53 Our results show that inulin and MCC increased the mRNA expression of caspase-1 by 177.13 and 134.97%, respectively, compared with the DSS group. The trends of inflammatory cytokine expressions when expressed at low levels.53 Our results show that inulin and MCC increased the mRNA expression of caspase-1 by 177.13 and 134.97%, respectively, compared with the DSS group. Inulin treatment resulted in the attenuation of this decrease (Figure 8A), indicating that the sequencing coverage was sufficient for subsequent analysis. As seen in Figure 8B,C, mice in the DSS group displayed a significantly reduced microbial diversity (Shannon index) and microbial richness (ACE index) compared with the control mice. However, administration of inulin, but not MCC, resulted in a significantly greater microbial diversity and richness compared with mice treated with DSS only, which were increased by 26.74 and 15.54%, respectively. As shown in Figure 8D, DSS and DF treatments changed the composition of the colon microbiota. The differences in colon microbiota composition between the four groups can also be intuitively confirmed by principal component analysis (PcoA) (Figure 8E). The results indicated that there was an obvious clustering relationship in the DSS group versus the control group with respect to the intestinal flora. The significant difference between the inulin group and the DSS group indicated that inulin had a regulatory effect on the microbial community composition of mice with DSS-induced colitis. Moreover, administration of MCC also resulted in a visible improvement of microbial community composition.

The cladogram, derived from the linear discriminant analysis effect size (LEfSe) analysis, showed a distinct gut microbiota composition for each of the four groups of mice (Figure 8F). The DSS treatment resulted in a significantly decreased relative abundance of *norank_f_Muribaculaceae* (Figure 8G), while inulin treatment resulted in the attenuation of this decrease with an abundance gain of 164.99% compared with the DSS group. *Muribaculaceae*, a novel name that includes members of the S24-7 family, contains a large number of multifunctional carbohydrate-active enzymes in the genome,59 which endow the host with the ability to digest DFs. The relative abundance of *Muribaculaceae* is negatively correlated with proinflamm-
tory cytokines and positively correlated with the expression of TJ proteins and MUC3. Compared with the control group, the abundance of Lactobacillus in the DSS group had also decreased significantly (Figure 8G). However, compared with the DSS group, in the MCC-administered group, a significant attenuation of the DSS-induced decrease in Lactobacillus was
observed, with the abundance of *Lactobacillus* increased by 167.96%. In a mouse model of colitis, *Lactobacillus paracasei* was found to reduce intestinal inflammation and expression of proinflammatory factors in the mucosa.\(^{53}\) Our finding that the relative abundance of *Lactobacillus* was decreased by DSS administration, which could, at least in part, be alleviated by MCC treatment is in agreement with Zhang et al.\(^{61}\) who found that MCC had a tendency to increase the relative abundance of *Lactobacillus* in the colon of mice, which may be related to the fact that MCC can promote the defection and bacterial community renewal of mice, reduce the adhesion of pathogenic bacteria, and indirectly increase the abundance of beneficial bacteria. The intestinal permeability and integrity of colitic mice can be broken by *Bacteroides*, while *Helicobacter* is thought to promote gastrointestinal disease.\(^{5,64}\) Here, we found that the relative abundance of *Bacteroides* and *Helicobacter* was the highest in the DSS group (Figure 8G). The administration of DFs (inulin and MCC) reduced the relative abundance of *Helicobacter* in colitic mice by 52.79 and 69.95%, respectively, compared with the DSS group, which might contribute to the reversal of the higher gut permeability and lower integrity of DSS-treated mice. A recent study found that bacteria from the relatively unknown *Turibacter* genus settled in the rumen and feces of cattle.\(^{65}\) Furthermore, the density of *Turicibacter* was correlated with the level of glycylproline, which can reduce the permeability of cefadroxil, a bactericidal antibiotic.\(^{53,66}\) Therefore, we speculated that *Turicibacter* might play a role in preventing toxic chemicals such as DSS to penetrate the intestinal barrier and induce an inflammatory response. Oral administration of inulin significantly (by 483.49%, \(p < 0.05\)) increased the abundance of *Turicibacter* compared with the DSS groups, and this might be related to a special function of inulin in maintaining gut health (\(p < 0.05\)). Significant attenuations of DSS-induced increase in *Escherichia-Shigella* were observed in inulin and MCC groups (Figure 8G), which were decreased by 97.37 and 63.07%, respectively, compared with the DSS group. Elevated expression of *Escherichia-Shigella* has been identified in the feces of UC patients and rectal biopsies of CD patients and is regarded as the microorganism that reinforces intestinal inflammation of IBD.\(^{65}\) A tendency to first rise and then fall of *Blautia*, *Alistipes*, *Mucispirillum*, and *Clostridium sensu stricto_1* was seen in the two DF groups, but there was no statistical difference between the DSS group with or without DFs (Figure 8G). In general, treatment with DFs can reverse, at least partially, the increase of colitis-with or without DFs (Figure 8G). In general, treatment with DFs (inulin and MCC) reduced the relative abundance of *Lactobacillus* in the colon of mice, which may be related to the fact that MCC can promote the defection and bacterial community renewal of mice, reduce the adhesion of pathogenic bacteria, and indirectly increase the abundance of beneficial bacteria. 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These results indicated that DFs can exert an anti-inflammatory effect by reducing the invasion of immune cells and suppressing the expression of proinflammatory cytokines in the colon.\(^{57}\) A possible mechanism for the beneficial effects of DFs, particularly fermentable fibers, could be that they promote the growth of beneficial bacteria and are metabolized into substances that have a range of anti-inflammatory and other beneficial effects in the host.\(^{66}\) Although MCC has a smaller particle size, a larger specific surface area, and better thermal stability than inulin, in most cases, inulin showed better protection against DSS-induced colitis than MCC. A possible reason is that inulin, as a soluble fiber, is easily fermented by microorganisms, can stimulate the colon bacteria to produce short-chain fatty acids (butyric acid and propionic acid), promote the growth of *Bifidobacterium* and *Lactobacillus*, and offset the proinflammatory activity of other known symbiotic bacteria (*Bacteroides* or *E. coli*),\(^{67}\) and therefore, compared with MCC, inulin is better at easing colonic mucosa inflammation.

### 3. CONCLUSIONS

MCC exhibited a smaller particle size, a larger specific surface area, and higher thermal stability than inulin. It exhibited anti-inflammatory activities by preventing weight loss and reducing the DAI of DSS-treated mice. Additionally, DFs enhanced the gut barrier function by repairing colon mucosa and reducing the invasion of immune cells, increasing the expression of TJ proteins, and regulating the number of probiotics in the colon. Moreover, DFs downregulated the proinflammatory cytokines. Therefore, we propose that DFs are promising functional additives for relieving intestinal inflammation. However, the exact metabolites produced by inulin or MCC and how they impact the host remain to be determined, and further work should characterize the metabolome of the intestinal contents in mice fed with different DFs to investigate the potential relationship between metabolism of DFs and gut flora.

### 4. MATERIALS AND METHODS

#### 4.1. Chemicals

DSS (molecular weight 36000–50000) was purchased from MP Biomedicals (CA, USA). Inulin and MCC were obtained from Runyan Trading Market Co., Ltd. in Zhengzhou. Primers were designed according to the gene sequences in GenBank and synthesized by Shenggong Co. (Zhengzhou, China). Hematoxylin and eosin (H&E) staining solution was purchased from Sigma Co. (MO, USA). All chemicals were of analytical grade.

#### 4.2. Particle Size Distribution.

The particle size and size distribution were measured with a Mastersizer-3000 (Malvern Instruments Ltd., Malvern, UK) analyzer. The monosaccharide composition was measured according to the method reported by Wang et al.\(^{68}\) with some modifications. Briefly, DFs (5 mg) were dissolved in trifluoroacetic acid (2 M) and hydrolyzed at 121 °C for 16 h. The residue was redissolved in deionized water and filtered through a 0.22 μm membrane, the supernatant of which was analyzed by high-performance anion-exchange chromatography on a CarboPac PA-20 anion-exchange column (3 × 150 mm, Dionex) using a pulsed amperometric detector (Dionex ICS 5000 system).

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#### 4.4. SEM.

The surface morphology of the DFs was investigated using a JSM-6390/LV SEM (NTC, Japan) microscope using a gold layer coating for 50 s after being sputter coated.

### Table 3. DAI Assessment

| score | weight loss (%) | stool consistency | stool blood |
|-------|-----------------|-------------------|-------------|
| 0     | none            | normal            | normal      |
| 1     | 1−5             | normal            | normal      |
| 2     | 5−10            | loose stools       | visible blood |
| 3     | 10−20           | loose stools       | visible blood |
| 4     | >20             | diarrhea           | rectal bleeding |

*DAI, disease activity index.*
dried to a constant weight. Images at 2000 X magnifications were obtained at an accelerating voltage of 15 kV.21

4.5. FT-IR. A Nicolet 6700 spectrometer (Nicolet Instrument Corporation, USA) was used to record FT-IR spectra in the range of 400–4000 cm⁻¹. Samples (2 mg) were dried and ground with KBr powder (spectroscopic grade) and then pressed into pellets (1 cm) for FT-IR analysis.22

4.6. XRD Analysis. XRD studies of DF samples were carried out using an X-ray diffractometer (Bruker AXS, Karlsruhe, Germany) with Cu Kα radiation (λ = 0.15418 nm) operating at a voltage of 40 kV and a current of 40 mA.30 The scanning speed was set at 1°/min, and the diffraction angle (2θ) ranged from 10 to 80°.

4.7. Thermal Analysis. DSC and TG were performed by a STA 449 C device (Netzsch, Selb, Germany) with a heating rate of 10 °C/min in a nitrogen environment.31 The DFs (about 15 mg) were weighed and heated from 20 to 300 °C. The nitrogen gas was injected into the device at the speed of 80 mL/min, and the data were recorded and analyzed using Netzsch software (Netzsch Inc., Selb, Germany).

4.8. Animals and Diets. All animal experiments were reviewed and approved by the Henan University of Technology IRB. A total of 32 female BALB/c mice (7 weeks old; weight 21 ± 0.5 g) were purchased from the laboratory animal center of Zhengzhou University. All experimental procedures were approved by the Animal Care and Use Committee of Henan University of Technology. The mice were housed under controlled temperature (22 °C), humidity (40–60%), and lighting (800–2000 lx) conditions. The mice were housed singly and acclimatized to the new environment while being fed an AIN-93 G diet and distilled water ad libitum for 1 week before formal experimentation began. The mice (n = 8) were randomly assigned to 4 groups: control, 4% (w/v) DSS (DSS group), 4% (w/v) DSS + 2.5 g/kg body weight inulin (inulin group), and 4% (w/v) DSS + 2.5 g/kg body weight MCC (MCC group). The dose selection of DFs was based on the World Health Organization’s recommended DF intake for normal adults.69 The control and DSS groups were fed the control diet, whereas the inulin and MCC groups were provided with diets containing inulin and MCC (both 2.5 g/kg body weight), respectively. Five days following the start of the experiment, the mice in the inulin and MCC groups were provided water containing 4% (w/v) DSS for 8 d, while the control groups were provided distilled water. Mice were assessed daily for the severity of colitis using a clinical score. At the end of the experiment, the mice were exsanguinated, and blood from the eyeballs and colon tissue was collected. The colons were dissected, and their weight and length were measured. Then, the colon tissues were quickly frozen in liquid nitrogen and used for qRT-PCR analysis. The MPO activity of colon tissues was analyzed using an MPO assay kit (JianCheng Bioengineering Institute, NanJing, China). Fresh colon contents were collected and were immediately stored at −80 °C to be later analyzed for microbiota using 16S rRNA gene sequencing.

4.9. H&E Staining. After the mice were sacrificed, colon tissues (8 μm) from the same area were harvested for H&E staining to observe the degree of intestinal injury and inflammatory cell infiltration.57 In brief, the samples were fixed in 10% neutral formalin solution for at least 24 h at room temperature following resection. The tissue sections were obtained by traditional techniques and were viewed with an Olympus CX41 microscope (Germany) at 200X magnification after staining with H&E.

4.10. DAI Assessment. The clinical scoring to assess the severity of colitis in mice is based on a standard scoring system according to Miles et al.60 In brief, the DAI score was recorded daily for all mice by assessing stool consistency, blood stool, and weight loss. Each score was determined as shown in Table 3. Bodyweight loss was calculated as the percentage of the difference between the initial body weight (day 0) and the weight on any given day.

4.11. qRT-PCR Analysis. The RNA was isolated from the colon tissue using the MiniBEST Universal RNA Extraction kit (Takara, Japan) following the manufacturer’s instructions. First strand cDNA was synthesized by using PrimeScript Reverse Transcriptase (Takara, Japan) during incubation at 42 °C for 15 min followed by deactivation at 85 °C for 5 s.

The one-step RT-PCR Kit (Toyobo, Japan) was used to perform qRT-PCR on a 7500 real-time PCR system (Applied Biosystems) under the following conditions: 94 °C for 30 s and then 40 cycles of 94 °C for 30 s followed by 60 °C for 30 s. The PCR primer sets for gene identification are listed in Table 4. The relative mRNA expression levels of genes were normalized to β-actin in each sample using the 2⁻ΔΔCT method with three replicates. Genes associated with intestinal barrier integrity (claudin-3, claudin-7, ZO-2, occludin, JAM-2, and MUC-3) and proinflammatory cytokines (caspase-1, NLRP3, TLR4, TNF-α, IL-1β) were detected in the colon tissue.

Table 4. Sequences of Primers Used for qRT-PCR

| target gene | forward (5′–3′) | reverse (5′–3′) |
|-------------|----------------|----------------|
| claudin-3   | CGGCTCGAGGCACTTTTGTTGACACCTTTTGGCG | GAAGATCTCCAGATGTTCCTAGGGCTAGT |
| claudin-7   | GAGTTGTGTCATGAGTGTGACATCA | TGGCAAGTCCAACTCTCAGT ACT |
| ZO-2        | CTTGTTGGAATGATGTTGCG | CCGGACTAATCTCCTGAAA |
| occludin    | TGCCATATGAGAGGGCTATGAG | AAGGAAGCGATGAGGACAGAAGC |
| JAM-2       | ATGCTGCTGGTCGCTACACTACTTT | TCTCGTCTGACATCCTACCTGAA |
| MUC-3       | CTTGTTGCAATCGGCTCAGAGTGG | AGTCCAAAGACAGCCGATATTCT |
| caspase-1   | CTGGGAGACATCCTGGACTGAG | GCCAGTCGAAAGGAGGTag |
| NLRP3       | CCATCAATGCGTTGCAGCA | AGATCCCACCTCCTCAGGGC |
| IL-1α       | CAACCAAGAATGATATTCCTCCATG | GAGGCTGGGAAAGGAGGAGG |
| TLR4        | GCTCTCAGCCCTCAGCCAAAG | ACGTCGACACTCTCCTCAG |
| TNF-α       | CTCTAGCACCACCATCAAGG | ACCCTGACACTCTCCTCAG |
| β-actin     | GTGAAAGTGCAGGCACTGGTTT | GAGAAGTGGGTGGTCTTGTAGGA |

ZO-2, tight junction protein 2; JAM-2, junctional adhesion molecule 2; MUC-3, mucins 3; NLRP3, NOD-like receptor protein 3; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor α.
4.12. DNA Extraction and Analysis of Microbiome. Proximal colonic contents were collected into a sterile centrifuge tube immediately after the mice were sacrificed for further 16S rDNA analysis according to Liu et al. In brief, the samples were stored at −80 °C until needed for DNA extraction. Aliquots (10 ng) of extracted DNA were used for amplifying and sequencing the V3−V4 hypervariable regions of the 16S rDNA. The operational taxonomic units (OTUs), ribosomal database project, fifth level of taxa assignment (genus), PCoA, and the distance matrix were analyzed on the online platform of Majorbio Cloud Platform (Majorbio Biopharm Technology Co., Ltd., Shanghai, China).

4.13. Statistical Analysis. All data are presented as mean ± SEM. Data of samples were compared using ANOVA with Duncan’s test (SAS version 9.2, SAS Inc., Cary, NC, USA). p < 0.05 indicated statistical significance.

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Notes
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■ ABBREVIATIONS

DFs, dietary fibers; IBD, inflammatory bowel disease; DSS, dextran sodium sulfate; MCC, microcrystalline cellulose; CD, Crohn’s disease; UC, ulcerative colitis; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TJs, tight junctions; LEISe, linear discriminant analysis effect size; SEM, scanning electron microscopy; FT-IR, Fourier-transform infrared spectroscopy; XRD, X-ray diffraction; TA, thermal analysis; DSC, differential scanning calorimetry; TG, thermo-gravimetry; MPO, myeloperoxidase; H&E, hematoxylin and eosin; DAI, disease activity index; E. coli, Escherichia coli; OTU, operational taxonomic units; RDP, ribosomal database project; PCoA, principal component analysis; NLRP3, NOD-like receptor protein 3; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor α

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