

Inulin hydrogel microspheres delivering catalase-modified probiotics for inflammatory bowel disease therapy

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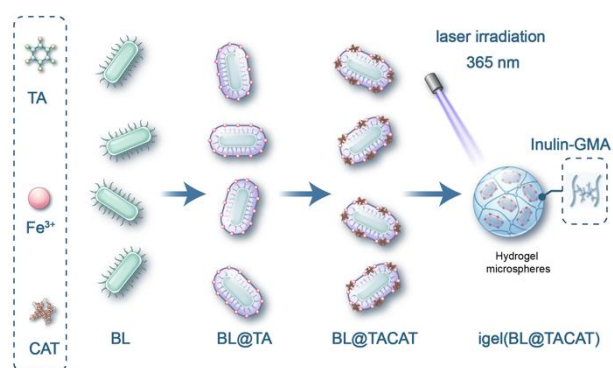
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Catalase- modified probiotics are encapsulated in inulin hydrogel microspheres. By mitigating intestinal oxidative stress and reshaping gut microbiota through inulin- derived prebiotic effects, the microspheres achieve promising therapeutic outcomes against inflammatory bowel disease.

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ABSTRACT: Probiotic-mediated gut microbiota regulation therapy holds great potential for the treatment of inflammatory bowel disease. However, harsh gastric conditions and persistent inflammation and oxidative stress in diseased intestines reduce probiotic survival and intestinal retention, severely compromising therapeutic efficacy. Herein, we fabricated inulin hydrogel microspheres encapsulating catalase-modified probiotics. This system alleviates the oxidative stress microenvironment in diseased intestinal tracts and regulates gut microbiota via the prebiotic effects of inulin, thereby achieving therapeutic effects against inflammatory bowel disease. First, the coordination complexation between tannic acid and ferric ions as well as intermolecular hydrogen bonds were utilized to stably immobilize catalase on the surface of probiotic bacteria. Subsequently, the modified probiotics were encapsulated within covalently crosslinked methacrylated inulin hydrogel microspheres via emulsion polymerization. Experimental results demonstrated that the functionalized microsphere system significantly mitigates intestinal oxidative injury, boosts the viability of probiotics under inflammatory stress, alleviates intestinal inflammation, reconstructs impaired intestinal barriers, and rebalances gut microbiota in animal models. Combining antioxidant modification with hydrogel microsphere delivery, this work establishes an innovative probiotic therapeutic platform, providing novel insights for adjuvant therapy of inflammatory bowel disease.

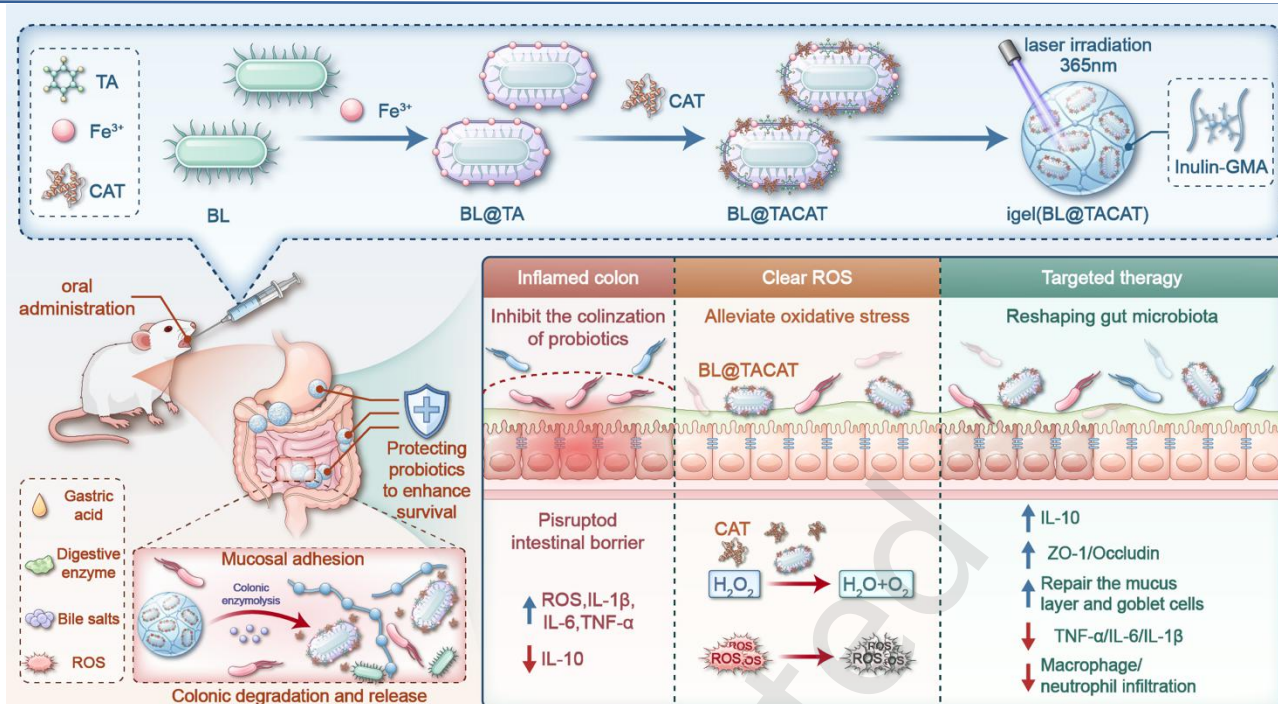
KEYWORDS: inflammatory bowel disease; hydrogel microspheres; probiotics; catalase; gut microbiota

1 Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), has become a major global public health concern, with both incidence and prevalence

continuing to rise over recent decades^[1-3]. The etiology and pathogenesis of IBD are highly complex and are generally believed to result from the interaction of multiple factors that lead to persistent and abnormal intestinal inflammation. These factors mainly include genetic susceptibility, immune dysregulation, gut microbiota imbalance, disruption of the intestinal mucosal barrier, and oxidative stress^[4-8]. Current clinical treatments primarily focus on suppressing abnormal immune-inflammatory responses to alleviate symptoms and improve patient outcomes^[9]. Although existing therapies have significantly improved the clinical prognosis of IBD patients, substantial limitations remain. Many patients exhibit poor responses to conventional medications, while adverse effects and concerns regarding long-term safety further restrict their clinical application^[10-13]. Therefore, despite the availability of numerous therapeutic approaches, IBD remains a significant medical challenge, highlighting the urgent need for more effective and safer treatment strategies.

Given the critical role of the gut microbiota in IBD pathogenesis, probiotic-based therapies aimed at restoring microbial homeostasis have attracted considerable attention in recent years^[14]. Compared with conventional drugs, probiotics possess the advantage of relatively low toxicity and fewer side effects^[15-17]. However, probiotic therapy also faces several limitations. The intestinal environment of patients with inflammatory bowel disease contains excessive levels of reactive oxygen species (ROS) and other harmful factors, which impair probiotic viability and functionality^[18, 19]. In addition, exposure to gastric acid, bile salts, and digestive enzymes significantly reduces probiotic survival, resulting in limited therapeutic efficacy when probiotics are administered alone^[20-23].



Scheme 1. Schematic diagram of preparation of inulin hydrogel microspheres loaded with catalase modified probiotic delivery system and treatment of inflammatory bowel disease.

Notably, excessive accumulation of hydrogen peroxide (H_2O_2), a major constituent of reactive oxygen species in the inflamed intestinal microenvironment, damages probiotic activity, disrupts the intestinal barrier, and continuously exacerbates inflammatory reactions^[24, 25]. To overcome these challenges, functional modification of probiotics has emerged as a promising research direction. In particular, endowing probiotics with antioxidant properties that enable active regulation of the local oxidative stress microenvironment has gained increasing interest^[26, 27]. Current strategies for bacteria-based IBD therapy primarily involve the use of nanozyme-modified probiotics to mimic the functions of antioxidant enzymes^[28-31]. Nevertheless, such nanozyme modification systems suffer from multiple drawbacks including biosafety risks, insufficient intestinal colonization capacity and unstable catalytic activity. Therefore, it is highly valuable to endow probiotics with the capacity to eliminate hydrogen peroxide by natural enzymes; however, the practical application of natural enzymes faces considerable challenges because they are susceptible to degradation and inactivation under harsh gastrointestinal conditions.

Catalase (CAT), an endogenous antioxidant enzyme, efficiently decomposes hydrogen peroxide into water and oxygen and plays a crucial role in maintaining redox homeostasis^[32, 33]. Incorporating catalase into probiotic systems may not only improve biosafety but also enhance probiotic survival under inflammatory conditions while simultaneously reducing oxidative damage to intestinal epithelial cells^[34]. Based on these considerations, *Bacillus licheniformis* was selected as the probiotic model owing to its ability to produce antimicrobial peptides, modulate the gut microbiota, and alleviate DSS-induced colitis. As shown in Scheme 1, a stable functional coating layer was constructed on the bacterial surface through the coordination interaction between tannic acid (TA) and ferric ions (Fe^{3+}), followed by immobilization of catalase to prepare antioxidant-functionalized probiotics (BL@TACAT). Tannic

acid is rich in phenolic hydroxyl groups and can bind strongly to proteins, polysaccharides, and other bacterial surface components through hydrogen bonding and electrostatic interactions, enabling stable adhesion to bacteria. It has been used for surface coating and enzyme/protein immobilization to preserve catalytic activity and protect biomolecules. Furthermore, tannic acid itself possesses intrinsic free-radical scavenging activity, which can partially alleviate oxidative stress-induced damage to probiotics^[35, 36]. Accordingly, we used the TA/ Fe^{3+} network to immobilize CAT on the surface of engineered probiotics, thereby improving its stability and maintaining its H_2O_2 -scavenging activity in the inflamed gut.

To further enhance probiotic stability during gastrointestinal transit and improve targeted release, an inulin hydrogel microsphere delivery system was employed to encapsulate the modified probiotics. Inulin is a natural fructan and also a soluble dietary fiber, with good oral safety and biocompatibility. It cannot be digested in the human small intestine but can be metabolized by colonic microbiota into short-chain fatty acids, thereby regulating gut microbial composition and suppressing intestinal inflammation. Based on these properties, inulin was selected as a carrier for delivering catalase-modified live probiotics to withstand the harsh gastrointestinal environment^[37, 38]. Self-assembled hydrogels derived from native inulin suffer from poor mechanical properties and tend to disintegrate in the gastrointestinal tract. Compared with native inulin, methacrylated inulin can rapidly covalently crosslink under mild conditions to form a stable crosslinked network, providing a robust physical barrier against gastric acid, bile salts, and digestive enzymes for probiotics and thereby improving their viability. More importantly, methacrylation occurs on the hydroxyl groups without altering the fructan backbone, thus preserving the intrinsic prebiotic activity of inulin. Based on this, we introduced methacrylate groups onto the inulin backbone, enabling the rapid formation of inulin hydrogel microspheres via

light-induced crosslinking and emulsion polymerization^[39-41]. The catalase-modified probiotics were subsequently encapsulated within these microspheres. Through both in vitro and in vivo experiments, the developed system was evaluated for its ability to alleviate oxidative stress, protect the intestinal barrier, and improve inflammatory conditions, thereby providing a novel therapeutic strategy for the treatment of inflammatory bowel disease.

2 Experimental

2.1 Materials

Tannic acid, glycidyl methacrylate (GMA), dimethyl sulfoxide (DMSO), N,N'-methylenebisacrylamide, and mineral oil were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Ferric chloride was obtained from Shanghai Yuanchuang Biotechnology Co., Ltd. (Shanghai, China). LB broth medium and LB agar medium were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Catalase, inulinase, lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP), and Span 80 were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Inulin was purchased from Sigma-Aldrich (Missouri, USA). Cell Counting Kit-8 (CCK-8), hydrogen peroxide assay kits, and 7'-dichlorodihydrofluorescein diacetate (DCFH-DA) ROS probes were purchased from Beyotime Biotechnology (Shanghai, China). The LIVE/DEAD™ BacLight™ Bacterial Viability Kit was purchased from Thermo Fisher Scientific. Dulbecco's Modified Eagle Medium (DMEM, high glucose), fetal bovine serum (FBS), and trypsin-EDTA solution was purchased from Gibco (Grand Island, NY, USA). HCT116 cells were obtained from the American Type Culture Collection (ATCC). Dextran sulfate sodium (DSS) was purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Commercial ELISA kits for IL-1 β , IL-6, IL-10, and TNF- α were obtained from MLBIO Biotechnology Co., Ltd. (Shanghai, China). Antibodies against myeloperoxidase (MPO), ZO-1, and Occludin were supplied by Shanghai Runer Biotechnology Co., Ltd. (Shanghai, China). All other chemicals used in this study were of analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2 Preparation of BL@TACAT

Bacillus licheniformis was cultured to the logarithmic growth phase and harvested by centrifugation. After washing with phosphate-buffered saline (PBS), the bacterial concentration was adjusted to 1×10^7 CFU/mL. Subsequently, 20 μ L tannic acid solution (3 mg/mL) and 20 μ L FeCl₃ solution (1 mg/mL) were sequentially added, with 10 seconds of vortex mixing between each addition to facilitate the formation of a tannic acid coating layer on the bacterial surface. Next, 20 μ L catalase solution (20 mg/mL) was added and vortexed for 10 seconds, allowing catalase to be immobilized onto the surface coating through multiple intermolecular interactions, yielding BL@TACAT.

2.3 Characterization of BL@TACAT : Particle Size, Zeta Potential Morphological Observation

Hydrodynamic diameter and zeta potential (ζ) were measured using a Zetasizer Nano-ZS instrument. Briefly, 1 mL of each formulation was mixed with 1 mL deionized water and analyzed in triplicate from three independent batches. The morphology and particle size of BL and BL@TACAT were characterized using transmission electron microscopy (TEM, JEM-F200,

Japan). Samples were deposited onto carbon-coated copper grids (200 mesh), allowed to adsorb for 2 min, and excess liquid was removed using filter paper. The samples were subsequently freeze-dried for 24 h using a freeze dryer (Scientz-12ND/C, China). Surface morphology was further observed using field-emission scanning electron microscopy (FE-SEM, Sirion 200) operated at an accelerating voltage of 3 kV.

2.4 Growth Characteristics of Modified Bacteria

To evaluate whether surface modification affected bacterial proliferation, BL (1×10^7 CFU/mL), BL@TA (1×10^7 CFU/mL), and BL@TACAT (1×10^7 CFU/mL) were serially diluted ten fold and spread onto LB agar plates. After incubation for 24 h, colony-forming units (CFUs) were counted. For growth curve analysis, bacterial suspensions were transferred into 96-well plates with an initial optical density at 600 nm of approximately 0.2 and incubated at 37°C with gentle shaking. OD600 values were measured every 15 min using a microplate reader for a total duration of 8 h.

2.5 Bacterial Survival Under Hydrogen Peroxide Stress

BL, BL@TA, and BL@TACAT (1×10^7 CFU/mL) were incubated in 1 mL of hydrogen peroxide solution (200 μ M) at 37°C for 2 h. Following treatment, 100 μ L of each bacterial suspension was spread onto LB agar plates. After incubation for 24 h, colony numbers were determined by plate counting.

2.6 Growth of BL@TACAT in the Presence and Absence of Hydrogen Peroxide

Two groups of BL@TACAT (1×10^7 CFU/mL) were incubated for 2 h under conditions with or without hydrogen peroxide (20 μ M). Subsequently, both groups were inoculated into 10 mL of fresh LB medium and cultured under standard conditions. Samples were collected at 0, 12, 24, 36, and 48 h, and bacterial viability was assessed by colony counting on LB agar plates.

2.7 Cell Culture

Human colorectal epithelial cells (HCT116) were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained in 6 cm culture dishes at 37°C in a humidified atmosphere containing 5% CO₂.

2.8 Cell Viability Assay

The culture medium HCT116 cells were seeded into 96-well plates at a density of 5000 cells per well and cultured for 12 h and was then replaced with fresh medium containing BL, BL@TA, or BL@TACAT. One group received 400 μ M hydrogen peroxide to induce oxidative stress, while the other group was cultured without hydrogen peroxide. After incubation for 24 h at 37°C and 5% CO₂, cell viability was evaluated using a Cell Counting Kit-8 (CCK-8). Briefly, the medium was removed, and cells were gently washed with sterile PBS. Serum-free medium containing CCK-8 reagent (10:1, v/v) was then added and incubated for an additional 2 h. Absorbance was measured at 450 nm using a BioTek Synergy H1 microplate reader. All experiments were performed in triplicate.

2.9 Intracellular Reactive Oxygen Species (ROS) Detection

Intracellular ROS levels were assessed using the fluorescent probe DCFH-DA. HCT116 cells were first treated with hydrogen peroxide (400 μ M) to induce oxidative stress and subsequently incubated with BL, BL@TA, or BL@TACAT to evaluate their protective effects. According to the manufacturer's protocol, cells were incubated with 10 μ M DCFH-DA at 37°C for 30 min. After incubation, cells were washed three times with

serum-free DMEM to remove excess dye. Fluorescence images were acquired using a laser scanning confocal microscope (LSCM, Zeiss LSM 900) with excitation and emission wavelengths of 488 nm and 525 nm, respectively.

2.10 Synthesis of Inulin-GMA

Two grams of inulin were dissolved in 40 mL dimethyl sulfoxide (DMSO). After complete dissolution, 1.9 g (15.5 mmol) of 4-dimethylaminopyridine (DMAP) and 4.4 g (31 mmol) of glycidyl methacrylate (GMA) were added. The reaction mixture was stirred at 50°C for 12 h. Subsequently, hydrochloric acid was added to neutralize DMAP, adjusting the pH to approximately 8.0. The reaction product was dialyzed for one week using a dialysis membrane with a molecular weight cutoff of 1 kDa. Finally, the purified product was freeze-dried to obtain methacrylated inulin (Inulin-GMA).

2.11 Preparation of Inulin-GMA Hydrogel

A total of 200 mg of freeze-dried Inulin-GMA was dissolved in 1 mL PBS. Subsequently, 5 mg of LAP photoinitiator was added, and the solution was exposed to 365 nm ultraviolet light for 1 min to form an Inulin-GMA hydrogel.

2.12 Rheological Characterization

The rheological properties of the hydrogel were evaluated using a HAAKE MARS III rheometer equipped with a 20-mm parallel-plate geometry. A sample volume of 200 μ L was used for each measurement. Time-sweep oscillatory tests were conducted by applying 10% strain for 60 s, followed by 100% strain for another 60 s at a constant angular frequency of 10 rad·s⁻¹. Frequency-sweep measurements were performed by varying the frequency from 0.1 to 100 rad·s⁻¹.

2.13 Preparation of BL@TACAT-Loaded Inulin Hydrogel Microspheres

BL@TACAT-loaded hydrogel microspheres were prepared by emulsion polymerization of methacrylated inulin. Specifically, 200 mg of Inulin-GMA was dissolved in 1 mL PBS containing BL@TACAT (10^8 CFU) and 5 mg LAP photoinitiator to form a hydrogel precursor solution. The precursor solution was added dropwise into 3 mL mineral oil containing 2% Span 80 under continuous stirring and exposed to 365 nm UV LED light at an intensity of 10 mW cm⁻² for 1 min at a distance of 10 cm at room temperature to induce crosslinking. The resulting microspheres were collected by centrifugation at $300 \times g$ for 2 min. After washing three times with PBS, the BL@TACAT-loaded hydrogel microspheres were resuspended in PBS for subsequent experiments.

2.14 Morphological and Size Analysis

The morphology of the hydrogel microspheres was observed using optical microscopy. The size distribution was determined by measuring the diameters of multiple microspheres.

2.15 Degradation of Inulin Hydrogel Microspheres

Hydrogel microspheres were incubated with inulinase (40 U/mL) at 37°C for either 4 h or 12 h. Following enzymatic degradation, samples were freeze-dried for 48 h and analyzed using scanning electron microscopy (SEM, Sigma 300) to evaluate morphological changes.

2.16 Microstructure of BL@TACAT-Loaded Hydrogel Microspheres

To investigate the internal microstructure, hydrogel microspheres were freeze-dried for 48 h using a freeze dryer (Scientz-12ND/C, China). The dried samples were examined using a Sigma 300 scanning electron microscope (Zeiss,

Germany) operated at 5 kV. For fluorescence imaging, catalase was labeled with fluorescein isothiocyanate (FITC), and the distribution of BL@TACAT within microspheres was observed using laser scanning confocal microscopy (LSCM, Zeiss LSM 900).

2.17 Bacterial Growth Curves After Microsphere Encapsulation

Hydrogel microspheres containing BL@TACAT were completely degraded using inulinase. The released bacteria were resuspended in LB medium until an OD600 of approximately 0.2 was reached. The bacterial suspension was transferred to 96-well plates and cultured at 37°C with gentle shaking. OD600 values were recorded every 15 min for 8 h to generate bacterial growth curves. Free BL and BL@TACAT served as controls.

2.18 Colony Counting Assay

After enzymatic degradation of the hydrogel microspheres, released bacteria were resuspended in LB medium. A volume of 100 μ L bacterial suspension was spread onto LB agar plates and cultured overnight. The number of colonies was subsequently counted. Free BL and BL@TACAT were used as control groups.

2.19 Cytocompatibility of Inulin Hydrogel Microspheres

HCT116 cells were seeded into 96-well plates at a density of 5000 cells per well and co-incubated with hydrogel extracts. After 24 h of incubation, the culture medium was removed and cells were gently washed with sterile PBS. Serum-free medium containing CCK-8 reagent (10:1, v/v) was then added and incubated for an additional 2 h. Cell viability was quantified by measuring absorbance at 450 nm using a BioTek Synergy H1 microplate reader. To further evaluate cell growth and viability, HCT116 cells were stained with a Live/Dead Cell Viability Assay Kit (Calcein-AM/PI) and incubated at 37°C for 15 min. Fluorescence images were captured using a laser scanning confocal microscope (LSCM, Zeiss LSM 900).

2.20 In Vitro Gastrointestinal Tolerance Assay

Equal amounts of free BL and hydrogel-encapsulated igel(BL@TACAT) were separately suspended in simulated gastric fluid (SGF, pH 2.0) or simulated intestinal fluid (SIF, pH 6.8) and incubated at 37°C. Enzyme-containing SGF and SIF were prepared according to the United States Pharmacopeia (USP) standards. At predetermined time points, hydrogel microspheres were enzymatically degraded using inulinase. Equal volumes of bacterial suspensions were collected, appropriately diluted, and plated onto LB agar. Following incubation at 37°C for 24 h, colony-forming units (CFUs) were quantified by plate counting. In parallel, bacterial viability was assessed using a Live/Dead bacterial staining kit and observed using laser scanning confocal microscopy (LSCM, Zeiss LSM 900). To further evaluate tolerance to the simulated gastrointestinal environment (GIF; SGF followed by SIF), igel(BL@TACAT) was first incubated in SGF for 3 h and subsequently transferred to SIF for 6 h. After enzymatic degradation of the microspheres, bacteria were resuspended in LB broth, and OD600 values were recorded every 15 min to construct bacterial growth curves.

2.21 Evaluation of Intestinal Targeting Ability

All animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* issued by the National Institutes of Health (NIH) and were approved by the Animal Ethics Committee of Shanghai Tenth People's Hospital. Male C57BL/6 mice (8–10 weeks old) were randomly divided

into three groups and orally administered BL, BL@TACAT, or igel(BL@TACAT), respectively. After oral administration at a dose of 1×10^7 CFU per mouse, animals were sacrificed at predetermined time points (6, 12, 24, and 48 h). Fluorescence imaging was performed using an AniView 100 in vivo imaging system (Guangzhou Bio-Light Biotechnology Co., China). Images were acquired using the GFP channel with an excitation wavelength of 488 nm and an emission wavelength of 520–530 nm, corresponding to the fluorescence spectrum of FITC.

2.22 Evaluation of Preventive and Therapeutic Effects in DSS-Induced Colitis

All animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* issued by the National Institutes of Health (NIH) and were approved by the Animal Ethics Committee of Shanghai Tenth People's Hospital. Eight-week-old female C57BL/6J mice were housed under specific pathogen-free (SPF) conditions and randomly divided into five groups ($n \geq 4$ per group): Control group, DSS group, BL group (1×10^8 CFU), BL@TACAT group (1×10^8 CFU), and igel(BL@TACAT) group (containing 1×10^8 CFU). In the preventive administration protocol, mice in each group were orally gavaged every two days throughout the entire experimental period. Except for the control group, all other groups received freshly prepared 3% DSS solution in drinking water for one week to induce colitis. After completion of the DSS treatment phase, all groups were returned to normal drinking water. In the therapeutic evaluation protocol, the same DSS-induced colitis model and grouping strategy were employed. After completion of the DSS exposure period, mice were returned to normal drinking water and continuously administered different formulations for 7 days. Body weight was measured daily. At the end of the experiment, mice were sacrificed for sample collection. Colon length was measured, and colon tissues were collected for histopathological examination. Serum samples were collected for cytokine analysis (TNF- α , IL-6, IL-1 β , and IL-10), and fecal samples were collected for 16S rRNA sequencing. In addition, qualitative fecal occult blood testing was performed using the o-tolidine method according to the manufacturer's instructions.

2.23 Histopathological Analysis

At the conclusion of the experiment, tissue samples were harvested and fixed in 4% paraformaldehyde. Samples were embedded in paraffin and sectioned into 5- μ m-thick slices. Sections were stained with hematoxylin and eosin (H&E) and examined under an optical microscope. For evaluation of mucus secretion, colon sections were stained using Alcian Blue–Periodic Acid–Schiff (AB-PAS) staining. Inflammatory cell infiltration was assessed by staining for myeloperoxidase (MPO).

2.24 Immunofluorescence Staining

Colon tissue sections were first incubated with primary antibodies, followed by incubation with HRP-conjugated secondary antibodies. Fluorescence microscopy was used to detect the expression of CD68, F4/80, ZO-1, and Occludin-1 in colon tissues.

2.25 Enzyme-Linked Immunosorbent Assay (ELISA)

To quantify inflammatory cytokines in mice, blood samples were centrifuged at 3000 rpm for 15 min to obtain serum. The concentrations of TNF- α , IL-6, IL-1 β , and IL-10 were measured using commercial ELISA kits according to the manufacturers' instructions.

2.26 In Vivo Biosafety Evaluation

On day 7 after treatment, major organs including the heart, liver, spleen, lungs, kidneys, and colon, were collected from treated mice for biosafety assessment. Healthy mice served as controls. Excised organs were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with H&E for histological examination. Serum samples were additionally collected for liver and kidney function analyses.

2.27 Hemolysis Assay

After diluting 1 mL of whole blood collected from mice with 2 mL of PBS, red blood cells (RBCs) were separated from serum by centrifugation at 2000 rpm for 10 minutes. Wash the red blood cell pellet four times with PBS and resuspend it in 10 mL PBS. Next, dilute 200 μ L of red blood cell suspension with 1 mL of PBS (negative control), deionized water (positive control), BL, BL@TA. The BL@TACAT And inulin hydrogel microsphere extract. After incubating at 37 ° C for 2 hours, centrifuge the mixture at 12000 rpm for 10 minutes. Transfer all supernatant to a 96 well plate and measure the absorbance at 570 nm using a multifunctional enzyme-linked immunosorbent assay (BioTek Synergy H1, USA). Finally, calculate the percentage of hemolysis of red blood cells according to the following formula:

$$\text{Hemolysis rate (\%)} = (OD_e - OD_s) / (OD_w - OD_s) \times 100\%$$

Among them, OD_e is the absorbance of the experimental group, OD_s is the absorbance of the negative control group, and OD_w is the absorbance of the positive control group.

3 Results and discussion

3.1 Preparation and characterization of catalase-modified probiotics

In the present work, *Bacillus licheniformis* was used as a model probiotic, and catalase was immobilized on the surface of the probiotic through the synergistic coordination of tannic acid and ferric chloride. Ferric ions (Fe^{3+}) coordinate with TA to assemble a robust metal-phenolic network shell wrapping the probiotic surface, onto which CAT molecules are anchored (**Fig. 1A**)^[42, 43]. Compared to the white deposition of native BL, the color of BL has changed to black purple (**Fig. S1**). TEM observations showed that BL@TACAT exhibited an obvious coating layer on the bacterial surface, with rough or granular structures appearing on the cell surface (**Fig. 1B**). Dynamic light scattering (DLS) measurements showed that the average hydrodynamic diameters of BL, BL@TA, and BL@TACAT were 755.33 ± 223.40 nm, 1156.16 ± 175.26 nm, and 1116.66 ± 153.41 nm, respectively (**Fig. 1C**). Notably, the particle size of BL@TACAT increased significantly, mainly due to the successful attachment of enzyme molecules to the surface of the metal-phenolic network. In addition, with the progression of modification, the zeta potential changed from -18.3 ± 0.7 mV to -22.1 ± 0.3 mV and finally to -26.1 ± 0.9 mV (**Fig. 1D**), indicating that the introduction of enzyme molecules affected the surface charge environment. Subsequently, catalase was labeled with FITC. Flow cytometry analysis showed that the fluorescence intensity of the BL@TACAT-FITC group shifted toward the high-intensity region, and the entire distribution curve shifted to the right (**Fig. 1E, F**). Similarly, measurements using a microplate reader also showed that the fluorescence intensity of the bacteria increased after modification (**Fig. S2**). Confocal laser scanning microscopy revealed a large amount of green fluorescence on

the surface of probiotics in the BL@TACAT group, indicating that FITC-labeled CAT was successfully modified onto the surface of the probiotics (**Fig. 1G**).

To determine whether catalase modification affected bacterial growth and proliferation, BL, BL@TA, and BL@TACAT were

cultured on LB agar plates for 12 h, and colony numbers were subsequently recorded (**Fig. 1H**). Plate counting results showed no obvious difference in colony numbers among the three groups (**Fig. 1I**). Subsequently, bacteria from each group were cultured in LB medium, and

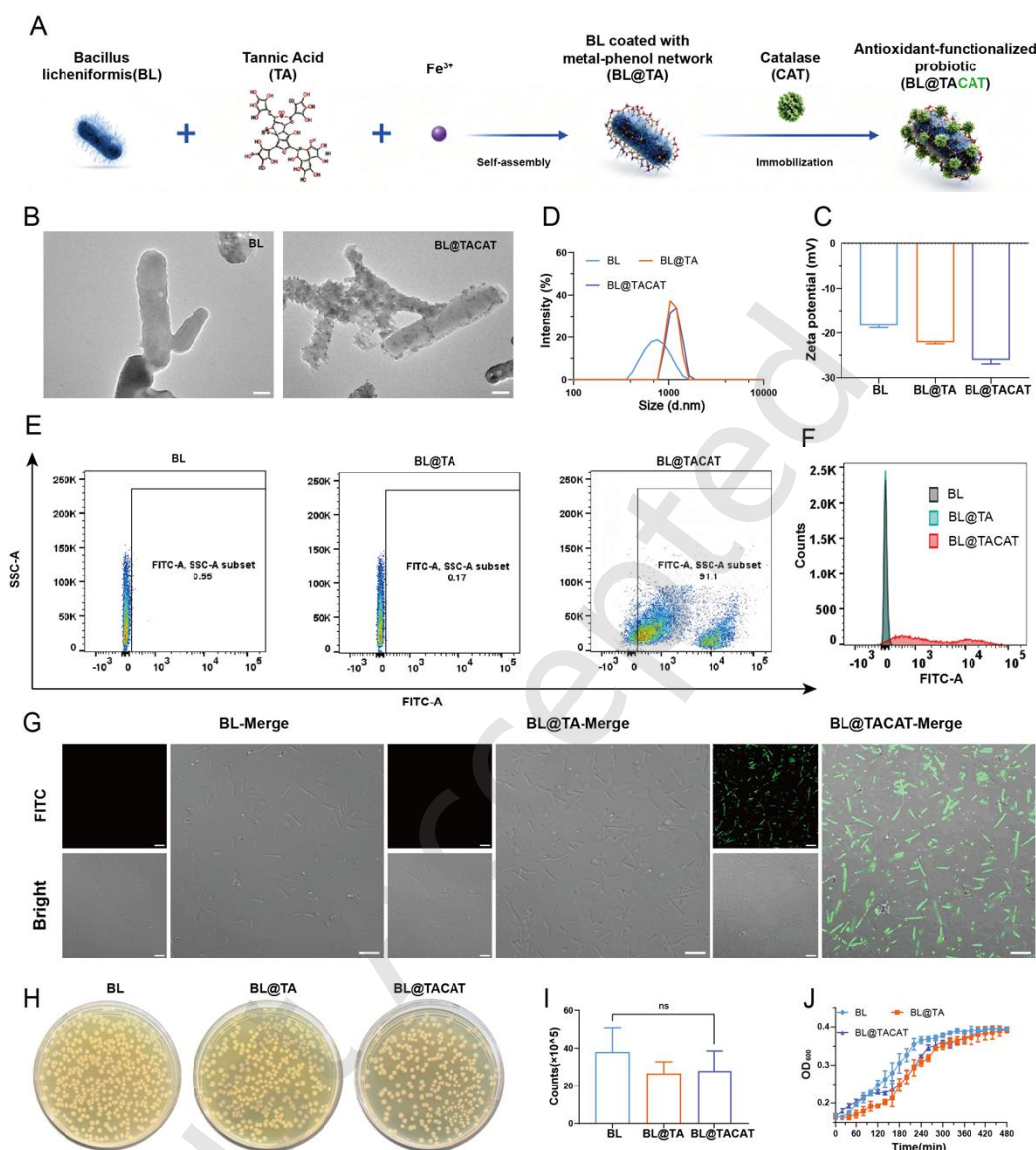


Figure 1 Preparation and Characterization of Inulin Hydrogel Microspheres Loaded with Catalase Modified Probiotic A) The preparation process of BL@TACAT. B) TEM images and C) zeta potential and D) size distribution of BL, BL@TA, BL@TACAT; scale bar: 500 nm. E), F) Flow cytometry and fluorescence distribution after FITC labeling. G) Confocal images of BL, BL@TA, BL@TACAT and the CAT was labeled with FITC, scale bac: 20 μm . H) Agar plate image of BL, BL@TA, BL@TACAT. I, J) colony count (I) and growth curve (J) of BL, BL@TA, BL@TACAT.

growth curves were monitored over 8 h (**Fig. 1J**). It could be observed that the growth rates of the three groups were essentially identical, indicating that the modification process had no significant inhibitory effect on bacterial growth.

3.2 In vitro antioxidant capacity of BL@TACAT

Excessive ROS play a key role in the pathogenesis of IBD. Hydrogen peroxide is a most common endogenous ROS and can be converted into highly toxic hydroxyl radicals. Catalase can efficiently catalyze the decomposition of hydrogen peroxide into water and oxygen and plays an important role in maintaining cellular redox balance^[44, 45]. Therefore, the antioxidant capacity of BL@TACAT was evaluated through in vitro experiments in this study.

First, a dissolved oxygen meter was used to record oxygen

generation after adding probiotics from different treatment groups into H_2O_2 solution. The oxygen generation rate of the BL@TACAT group increased rapidly, and the oxygen concentration reached 5.9 mg/L within 5 min, whereas that of the BL group was only 0.79 mg/L, indicating that BL@TACAT could continuously catalyze the decomposition of H_2O_2 and generate H_2O and O_2 (**Fig. 2A**). Subsequently, the H_2O_2 clearance rate was measured. The results showed that the H_2O_2 scavenging efficiency of BL@TACAT was approximately five times higher than that of the unmodified BL group, indicating its excellent ability to decompose hydrogen peroxide (**Fig. 2B**).

To evaluate the survival ability of BL@TACAT under a hydrogen peroxide environment, BL, BL@TA, and BL@TACAT were added to 200 μM H_2O_2 and incubated at

37°C for 2 h, followed by plate counting. The plate counting results showed that the BL@TACAT group still maintained a high survival rate, indicating that CAT could protect probiotics from the effects of a hydrogen peroxide environment (Fig. 2C and Fig. S3). In addition, BL@TACAT was cultured under conditions with and without H₂O₂, and bacteria were collected at

different time points for plate counting to observe bacterial growth. The results showed that BL@TACAT could grow normally in the absence of H₂O₂, and its proliferation was barely inhibited under H₂O₂ exposure, indicating strong tolerance to oxidative stress (Fig. 2D, E).

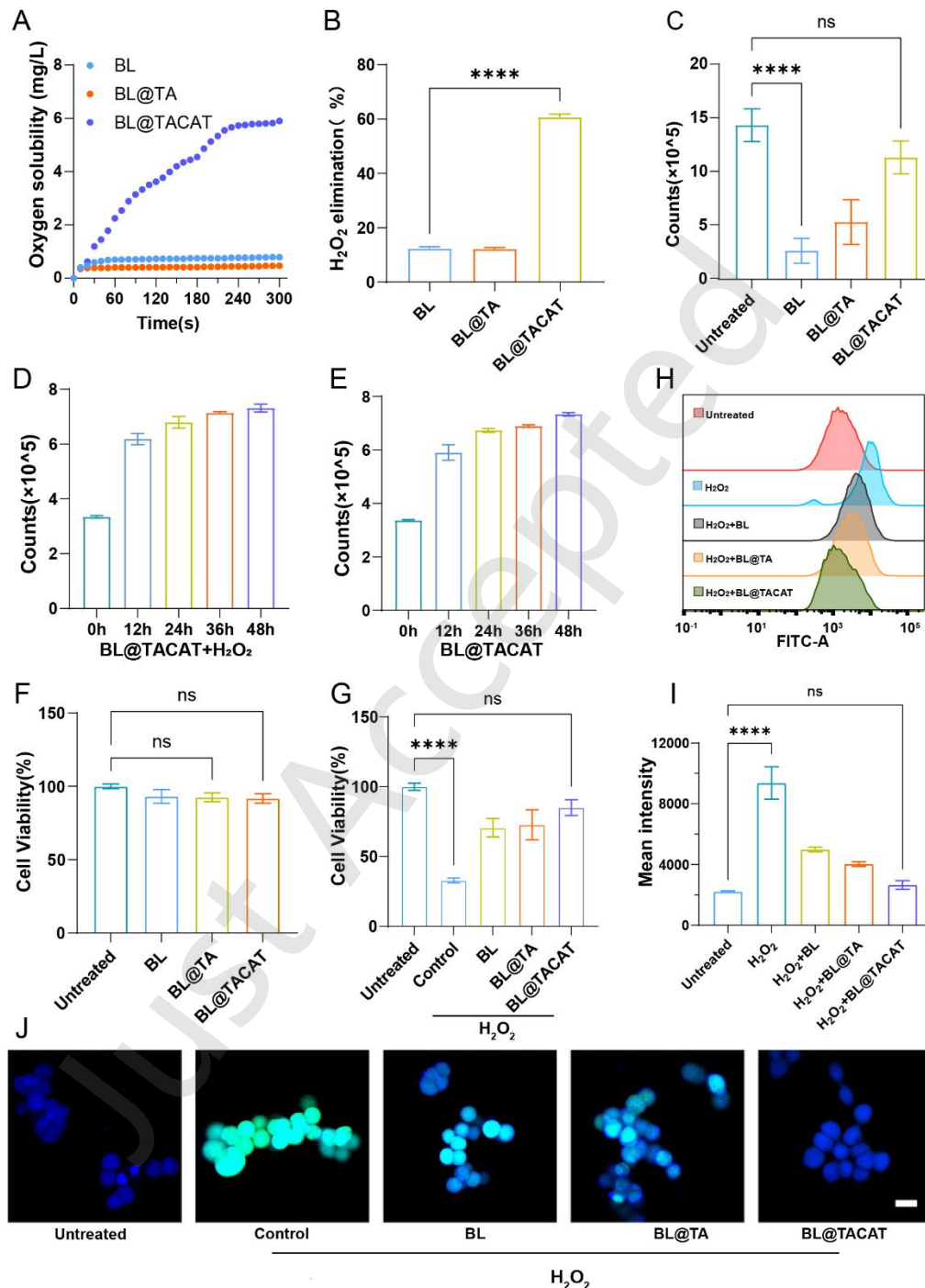


Figure 2 In vitro antioxidant activity of Inulin Hydrogel Microspheres Loaded with Catalase Modified Probiotic. A) Oxygen generation rate of BL, BL@TA, BL@TACAT. B) H₂O₂ elimination of BL, BL@TA, BL@TACAT. C) Colony counts after hydrogen peroxide incubation. D), E) Proliferation of BL@TACAT in the absence (D) or presence (E) of 400 μM H₂O₂ for different periods of time. F) Relative cell viability of HCT116 cells with various treatments. G) Relative viability of HCT116 cells with various treatments in the presence of 400 μM H₂O₂. H, I) Flow cytometry histogram and Positive percent after H₂O₂ (400 μM) treatments stained with DCFH-DA. J) Confocal fluorescent images of ROS levels in HT29 cells with different treatments, scale bar: 20 μm.

Based on the excellent in vitro antioxidant capacity of BL@TACAT, its antioxidant performance was further evaluated at the cellular level. First, the cytocompatibility of the modified

bacteria was assessed using the CCK-8 assay. The results showed that, in the absence of hydrogen peroxide, the cell viability in all treatment groups was comparable to that of the

control group, indicating that the modified bacteria themselves possessed good biocompatibility (**Fig. 2F**). After the addition of hydrogen peroxide, cell viability in the control group decreased markedly, whereas treatment with BL@TACAT restored cell viability, demonstrating that the catalase immobilized on the bacterial surface could effectively alleviate oxidative damage (**Fig. 2G**). Subsequently, to further investigate the effect of BL@TACAT on intracellular ROS levels, HCT116 cells were

stained with the ROS-specific fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Flow cytometry analysis confirmed these results, demonstrating that BL@TACAT possessed strong ROS elimination capability (**Fig. 2H,I**). In addition, as shown in the fluorescence images (**Fig. 2J**), intracellular fluorescence signals were reduced after BL@TACAT treatment, indicating that it possessed ROS scavenging ability.

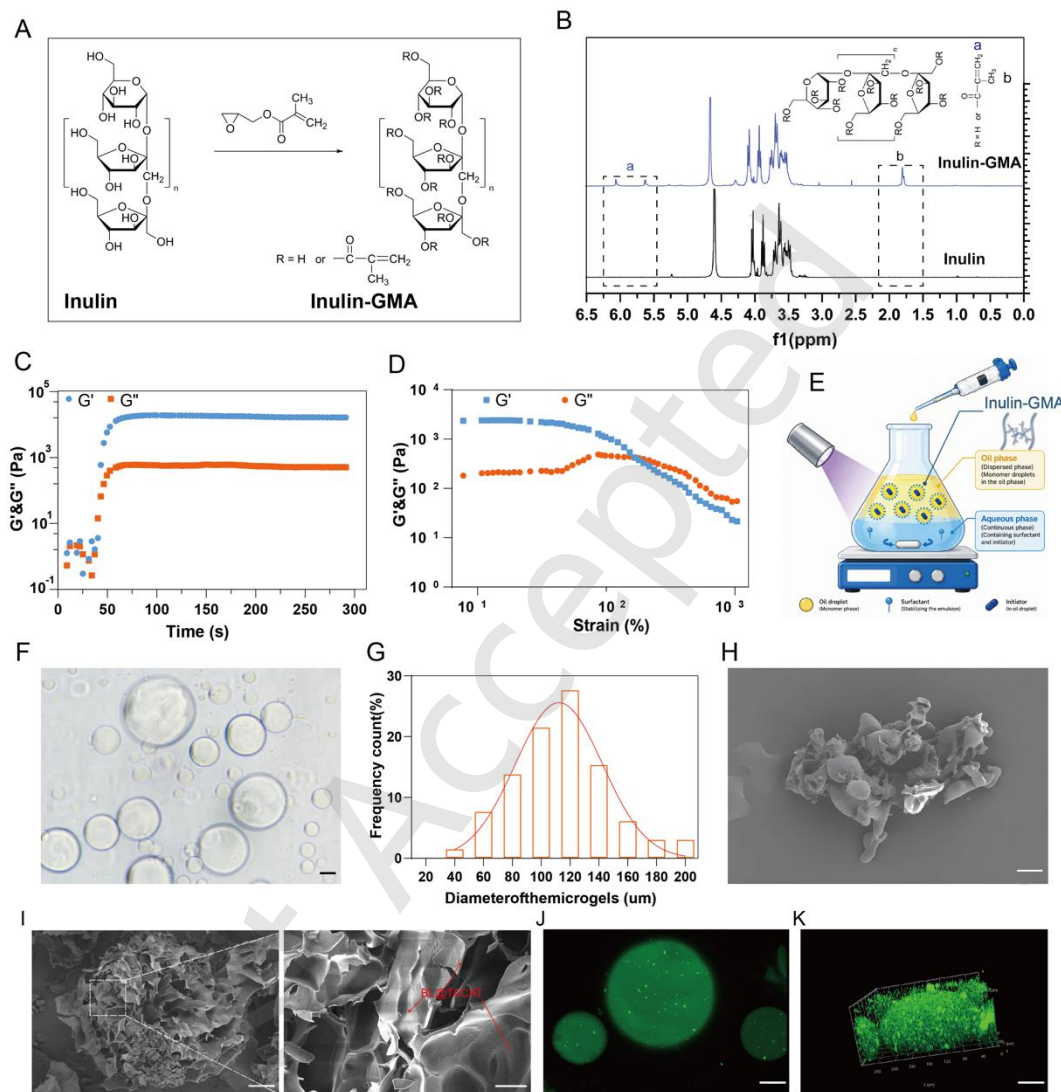


Figure 3 Preparation and Characterization of Inulin Hydrogel Microspheres. A) The synthetic route of Inulin-GMA. B) ^1H NMR data of Inulin-GMA. C) Test of gelation time of hydrogel. D) Strain-sweep oscillatory tests of hydrogel. E) Schematic diagram of inulin hydrogel microspheres formed by emulsion polymerization. F) Microscopic image of inulin hydrogel microspheres formed by emulsion polymerization. G) Particle size distribution of inulin hydrogel microspheres. H) Scanning images of inulin gel exposed to inulin external enzyme for 4 h at 37 °C; scale bar: 50 μm . I) SEM image of igel(BL@TACAT) and its internal BL distribution. Scale bar: 30 μm (left), 10 μm (right). J) After culturing 24 h in specifically LB medium, the images of bacteria wrapped in microspheres taken by laser confocal microscope and K) 3D mode, scale bar: 50 μm .

3.3 Construction, Characterization and Biocompatibility of Inulin Hydrogel Microspheres

To fabricate hydrogel microspheres with stable mechanical properties, methacrylate groups were grafted onto inulin. The modified inulin (Inulin-MA) was then subjected to emulsion polymerization with photoradiation-initiated crosslinking to form the hydrogel microspheres. The synthesis of Inulin-MA was verified by ^1H nuclear magnetic resonance (^1H NMR). Compared with inulin, Inulin-MA exhibited two additional peaks corresponding to chemical shifts at 6.1 and 5.3 ppm, which were characteristic signals of the GMA vinyl protons,

while the methyl peak at approximately 1.8 ppm also indicated that GMA had been successfully grafted onto inulin (**Fig. 3A and 3B**). To investigate the crosslinking behavior of Inulin-MA in the presence of photoinitiator, light-induced rheological tests were carried out on Inulin-MA solutions incorporated with LAP to characterize the variations in their rheological properties. Rheological test results show that during light irradiation, the storage modulus G' of the system rapidly surpasses the loss modulus G'' and remains constant, verifying the construction of a robust three-dimensional hydrogel network within the system (**Fig. 3C**). Furthermore, when the strain ranged from 0 to 100%,

the storage modulus (G') and loss modulus (G'') remained relatively stable, and G' was greater than G'' (Fig. 3D),

indicating that the hydrogel exhibited a certain degree of elasticity and structural stability.

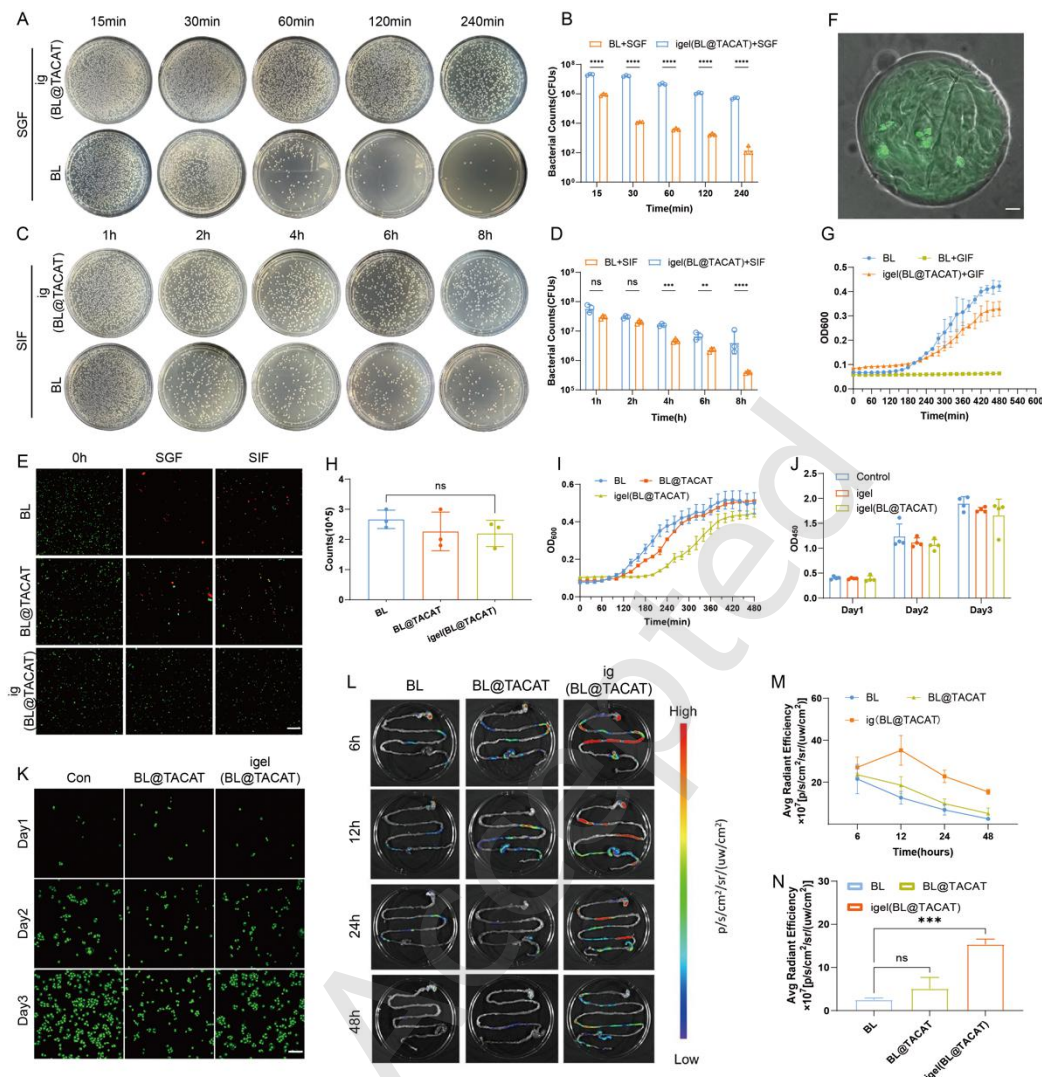


Figure 4 Resistance of hydrogel-encapsulated probiotics against gastrointestinal assaults and its enhanced retention in mouse intestine. A) Photographs of agar plates culturing BL or igel(BL@TACAT) bacteria. after incubation with SGF (pH = 2) for different time periods. B) Quantitative analysis of CFU on the agar plates after SGF incubation. C) Photographs of agar plates culturing BL or igel(BL@TACAT) bacteria after incubation with SIF (pH = 6.8) for different time periods. D) Quantitative analysis of CFU on the agar plates after SIF incubation. E) Live/dead bacterial viability assay of BL, BL@TACAT, and igel(BL@TACAT) before and after incubation in SGF for 2 h or SIF for 6 h. Scale bar: 50 μ m. F) Confocal image of igel(BL@TACAT) after incubation in SGF for 2 h and SIF for 6 h. G) The growth curve of BL, BL@TACAT, and igel(BL@TACAT) after incubation in SGF for 2 h and SIF for 6 h. H, I) BL@TACAT plate counting (H) and growth curve (I) after enzymatic hydrolysis by inulinase. J) HCT116 cells viability after treatment with different preparations for 3 days. K) Live/dead staining fluorescence imaging of HCT116 cells after incubation with PBS, BL@TACAT, igel(BL@TACAT). Scale bar: 100 μ m. L) The distribution of BL, BL@TACAT, and igel(BL@TACAT) (BL is labeled with green fluorescent (FITC) in the GIT for 48 h was investigated by intestinal transit assay. M) Quantification evaluation of bioluminescence signals of BL, BL@TACAT, and igel(BL@TACAT). N) Quantification of fluorescence intensity after 48 hours of residence in the intestine.

Subsequently, inulin-MA hydrogel microspheres were prepared through emulsion polymerization, which offers mild conditions, simple operation, good reproducibility, and easy scale-up, enabling efficient encapsulation of live bacteria and catalase while preserving bacterial viability and catalase activity. Thus, the modified probiotics were encapsulated within the hydrogel microspheres (Fig. 3E). Optical microscopy showed that the microspheres were spherical and uniform in size (Fig. 3F).

Statistical analysis of particle size distribution showed that the diameters of the inulin hydrogel microspheres were mainly distributed in the range of 80–160 μ m (Fig. 3G). After treatment with inulinase, scanning electron microscopy (SEM) revealed obvious structural collapse of the microspheres (Fig. 3H), demonstrating that the inulin hydrogel microspheres could be degraded and utilized in the colon to release

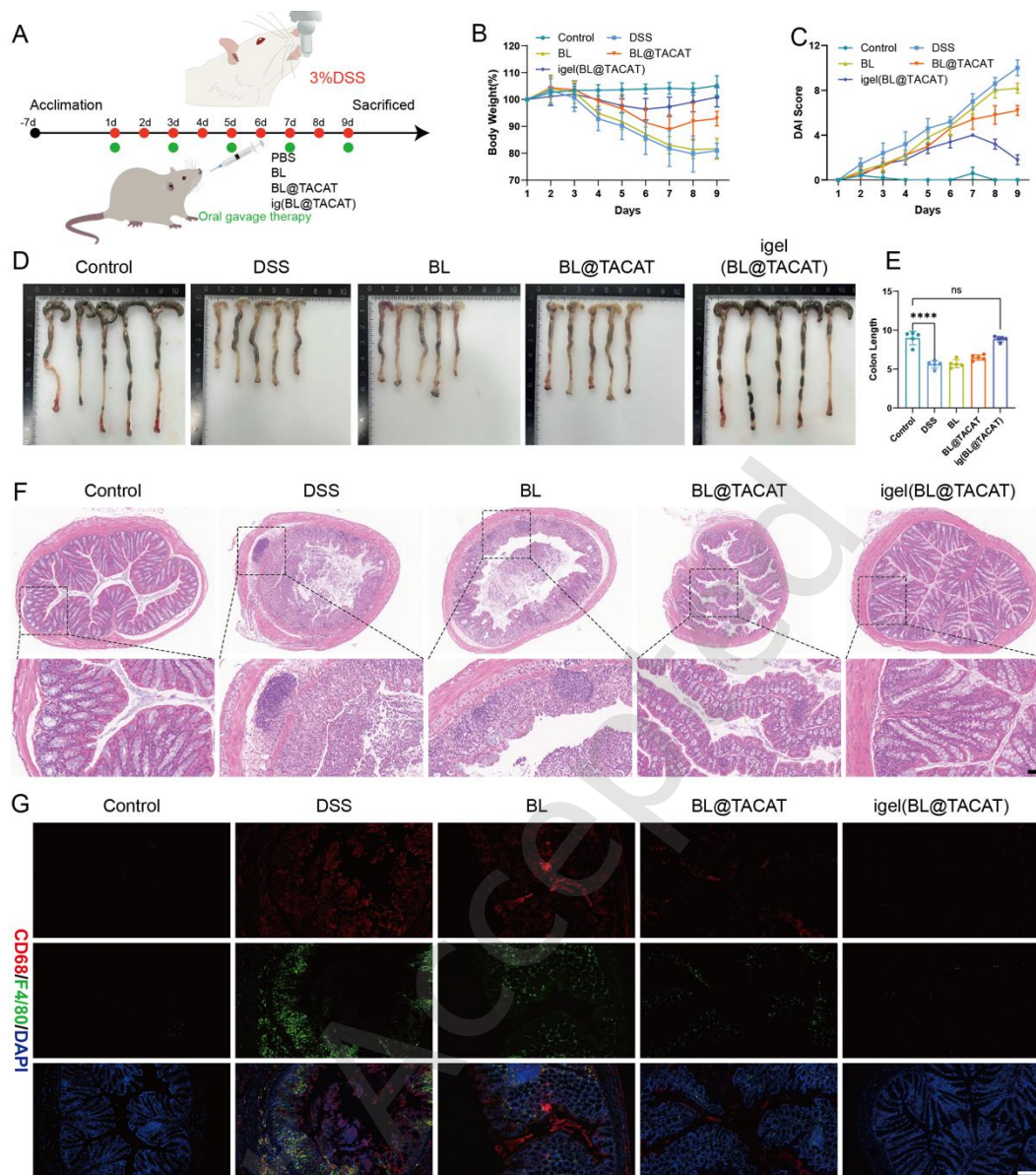


Figure 5 Prophylactic efficacy of igel(BL@TACAT) in a DSS-induced mouse colitis model. A) Schematic representation of the administration schedule in a DSS-induced acute colitis model. B) Body weight changes of mice subjected to different treatments ($n = 5$). C) Disease activity index (DAI) changes during treatment ($n = 5$). D, E) Colon tissue images and corresponding quantified colon lengths after various treatments ($n = 5$). F) Representative H&E-stained images of colon tissues in different groups. Scale bar: 100 μm . G) Representative immunofluorescence images of F4/80- and CD68-positive cells in colon tissue sections in different groups. Scale bar: 100 μm .

modified probiotics and exert therapeutic effects. Next, SEM was used to observe the microstructural characteristics of the crosslinked hydrogel. The results showed that the hydrogel possessed a spherical porous structure, and the probiotics encapsulated within the microspheres could be clearly observed (**Fig. 3I**). Furthermore, catalase on the surface of BL@TACAT was labeled with fluorescein isothiocyanate (FITC) and then encapsulated into inulin hydrogel microspheres. The distribution of probiotics within the microspheres was observed by confocal laser scanning microscopy (**Fig. 3J**). BL@TACAT was found to be uniformly distributed throughout the hydrogel microspheres, and three-dimensional imaging showed strong fluorescence signals from BL@TACAT embedded inside the microspheres (**Fig. 3K**), further confirming the successful encapsulation of BL@TACAT within the hydrogel microspheres.

3.4 Gastrointestinal Resistance, Biocompatibility, and Enhanced Intestinal Colonization of igel(BL@TACAT)

Probiotics are susceptible to damage from gastric acid, digestive enzymes, and ROS in the inflammatory intestinal environment, resulting in reduced survival and weakened therapeutic efficacy. To evaluate the protective effect of the hydrogel microspheres on modified probiotics, unmodified probiotics (BL) and BL@TACAT-encapsulated inulin hydrogel microsphere were separately resuspended in simulated gastric fluid (SGF, pH = 2.0) and simulated intestinal fluid (SIF, pH = 6.8), followed by incubation at 37°C. Samples were collected at different time points, and colony-forming units (CFUs) were determined by plate counting to assess probiotic survival under different treatment

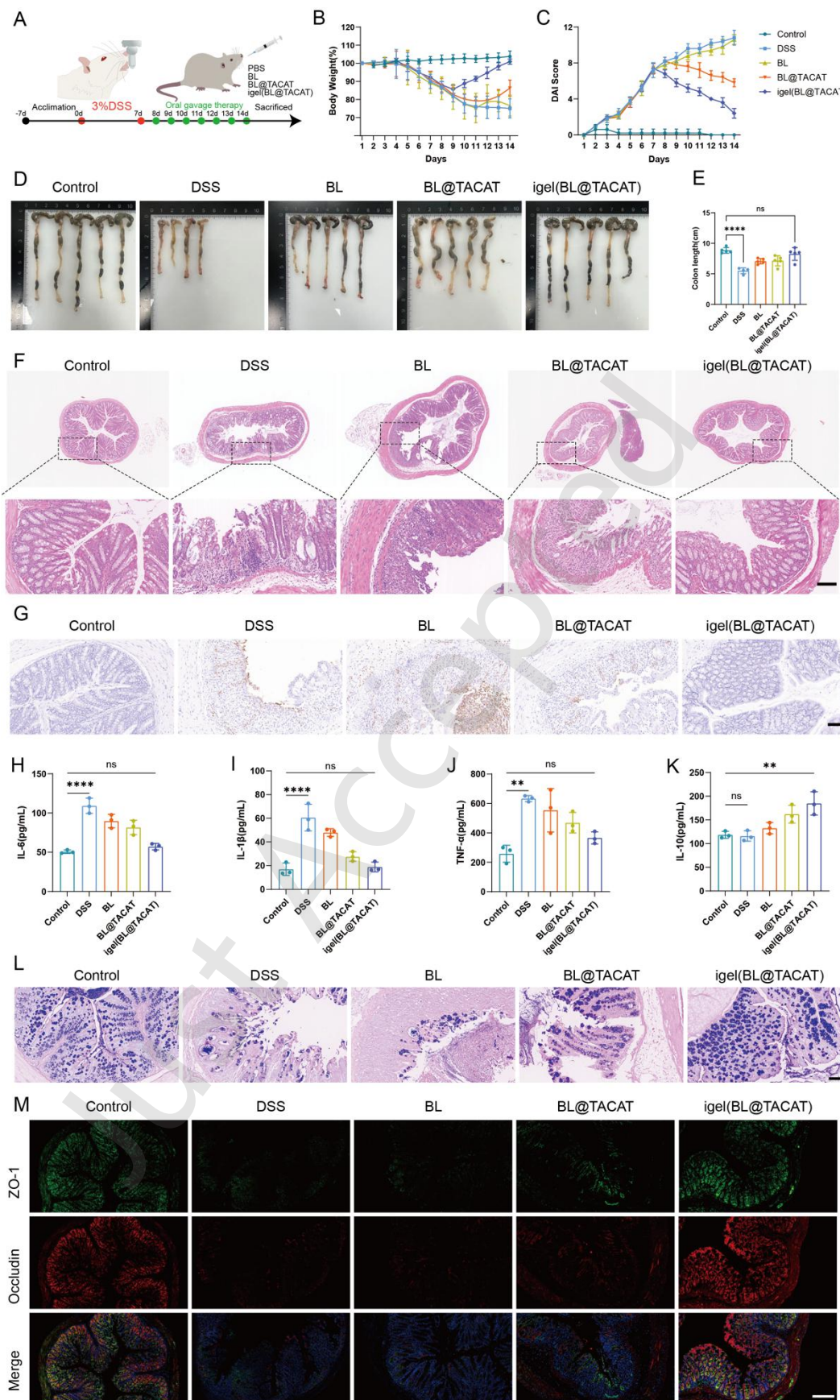


Figure 6 Therapeutic efficacy of igel(BL@TACAT) in a DSS-induced mouse colitis model. A) Schematic representation of the therapeutic evaluation procedure for igel(BL@TACAT) in a DSS-induced colitis model. B) Body weight variations in mice subjected to different treatments. C) Changes in disease activity index (DAI) during treatment. D, E) Colon tissue images and corresponding quantified colon lengths after various treatments. F) H&E-stained histological images of colon tissues in different groups. Scale bar: 100 μ m. G) Immunohistochemical analysis of myeloperoxidase (MPO) expression in the colon tissue sections. H-K) Serum concentrations of pro-inflammation cytokines (IL-1 β , IL-6, TNF- α) and anti-inflammation cytokine (IL-10) in each group were measured by the enzyme-linked immunosorbent assay (ELISA) kit. Data is expressed as mean $\pm$ SD. L) Representative images of AB-PAS staining in different groups. Scale bar:

100 μm . M) Representative immunofluorescence images of Occludin (red) and ZO-1 (green) with DAPI (blue) nuclear counterstaining. Scale bar: 100 μm .

Under simulated gastric acid conditions, the survival rate of igel(BL@TACAT) was higher than that of normal bacteria (**Fig. 4A, B**). In simulated intestinal fluid, the survival rate of igel(BL@TACAT) was also improved (**Fig. 4C, D**). Further live/dead bacterial staining also demonstrated that encapsulation within inulin hydrogel microspheres improved the survival of probiotics in the gastrointestinal tract (**Fig. 4E**). Subsequently, the survival status of igel(BL@TACAT) was investigated after treatment in SGF for 3 h followed by SIF for 6 h. Confocal microscopy showed that the microspheres still maintained an intact morphology, while the green fluorescence signals of bacteria within the microspheres further confirmed bacterial survival (**Fig. 4F**). Growth curves of bacteria released after enzymatic degradation of the microspheres also showed that the growth activity of igel(BL@TACAT) was increased, whereas BL without microsphere encapsulation almost completely lost its growth activity (**Fig. 4G**). These results demonstrated the protective capability of the inulin hydrogel microspheres toward BL@TACAT.

Next, the biocompatibility of the inulin hydrogel microspheres was evaluated. The hydrogel microspheres were degraded using inulinase to release the encapsulated BL@TACAT bacteria. Through bacterial plate counting (**Fig. 4H**) and growth curve (**Fig. 4I**) analysis, it was found that the growth and proliferation activities of BL@TACAT were not significantly affected. Subsequently, HCT116 cell proliferation after co-incubation with hydrogel extracts was evaluated using a CCK-8 assay. The results showed that the hydrogel had no obvious effect on cell viability (**Fig. 4J**), and live/dead cell staining further confirmed this finding (**Fig. 4K**). These results demonstrated that the inulin hydrogel microspheres possessed good biocompatibility. Moreover, after 7 days of oral igel(BL@TACAT) administration, major organs were harvested and subjected to H&E staining to evaluate systemic safety. The results showed that no pathological abnormalities were observed in any treatment group compared with the healthy control group (**Fig. S4A**). In addition, markers of liver and kidney toxicity, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CREA), were measured (**Fig. S4B-E**). No significant changes were observed in any treatment group, indicating that igel(BL@TACAT) exhibited no hepatotoxicity or nephrotoxicity. Furthermore, in vitro hemolysis assays showed that erythrocytes treated with igel(BL@TACAT) exhibited no obvious hemolysis, with a hemolysis rate close to 0%, further confirming the excellent biosafety of the material (**Fig. S5**).

The targeting and adhesion abilities of probiotics in the colon play important roles in their therapeutic efficacy. Therefore, FITC-labeled BL, BL@TACAT, and igel(BL@TACAT) were orally administered, and fluorescence imaging was performed at different time points (6, 12, 24, and 48 h) using an in vivo imaging system (**Fig. 4L**). The results showed that, at all-time points, the fluorescence intensity of the igel(BL@TACAT) group was higher than that of the other two groups (**Fig. 4M, N**), indicating stronger intestinal adhesion. This phenomenon may be attributed to the enhanced gastrointestinal mucosal adhesion provided by methacrylate-modified inulin.

3.5 Preventive Effect of igel(BL@TACAT) on DSS-Induced Colitis

Based on the promising in vitro results, the preventive efficacy of igel(BL@TACAT) was further evaluated in a dextran sulfate sodium (DSS)-induced mouse model of colitis. Eight-week-old female C57BL/6J mice were randomly divided into five groups ($n \geq 5$ per group): Control group, DSS group, BL group (1×10^8 CFU), BL@TACAT group (1×10^8 CFU), igel(BL@TACAT) group (containing 1×10^8 CFU). All groups, except the control, received 3% DSS in drinking water for 1 week to induce colitis. Each group received oral gavage of the corresponding formulation every two days, while the control and DSS groups were given saline. Subsequently, the preventive efficacy was evaluated through multiple indicators such as body weight, Disease Activity Index (DAI), colon length, and histological assessment.

As shown in **Fig. 5B** and **Fig. 5C**, all groups exhibited similar body weight trends during the first three days. From the fourth day onward, mice in the DSS group and BL group showed significant and continuous body weight loss. Although mice in the BL@TACAT group also experienced weight loss, the rate and extent of reduction were slower. In contrast, body weight loss in the igel(BL@TACAT) group was not obvious, and body weight began to increase after day six (**Fig. S6A**). The DAI results similarly demonstrated that igel(BL@TACAT) could prevent symptoms such as body weight loss and fecal bleeding caused by inflammatory bowel disease (**Fig. S6B**). Colon length in DSS-treated mice was reduced by 37.6% compared with the control group, whereas colon length in the igel(BL@TACAT) group was reduced by only 1.3% (**Fig. 5D, E**). In addition, the degree of colonic edema was alleviated after treatment, indicating a remarkable preventive effect against colitis.

Next, histological analysis of colon tissues from IBD mice was performed using hematoxylin and eosin (H&E) staining. The colon tissues of mice in the control group exhibited intact tissue architecture, orderly epithelial cell arrangement, uniform glandular organization, and no obvious inflammatory response. In contrast, DSS-treated mice showed significant structural damage and inflammation, characterized by extensive inflammatory cell infiltration and severe crypt destruction. The BL group and BL@TACAT group showed no obvious improvement in colon structural damage, indicating that probiotics not encapsulated within inulin hydrogel microspheres exhibited limited therapeutic efficacy. In the igel(BL@TACAT)-treated group, however, morphological damage to colon tissues was improved (**Fig. 5F**). Compared with the DSS group, glandular arrangement in the colon tissues of the igel(BL@TACAT)-treated group was more orderly, inflammatory cell infiltration was reduced, crypt morphology returned to normal, and intestinal structural integrity was restored.

To further evaluate the effect of preventive administration of igel(BL@TACAT) on colonic immune homeostasis in mice, immunofluorescence analysis was performed to examine the expression of CD68 and F4/80 in colon tissues. CD68 and F4/80 are macrophage markers that can be used to observe immune cell distribution and macrophage activation in the IBD model. Obvious CD68-positive and F4/80-positive staining areas were observed in DSS-treated mice, indicating macrophage

infiltration and activation. In contrast, CD68-

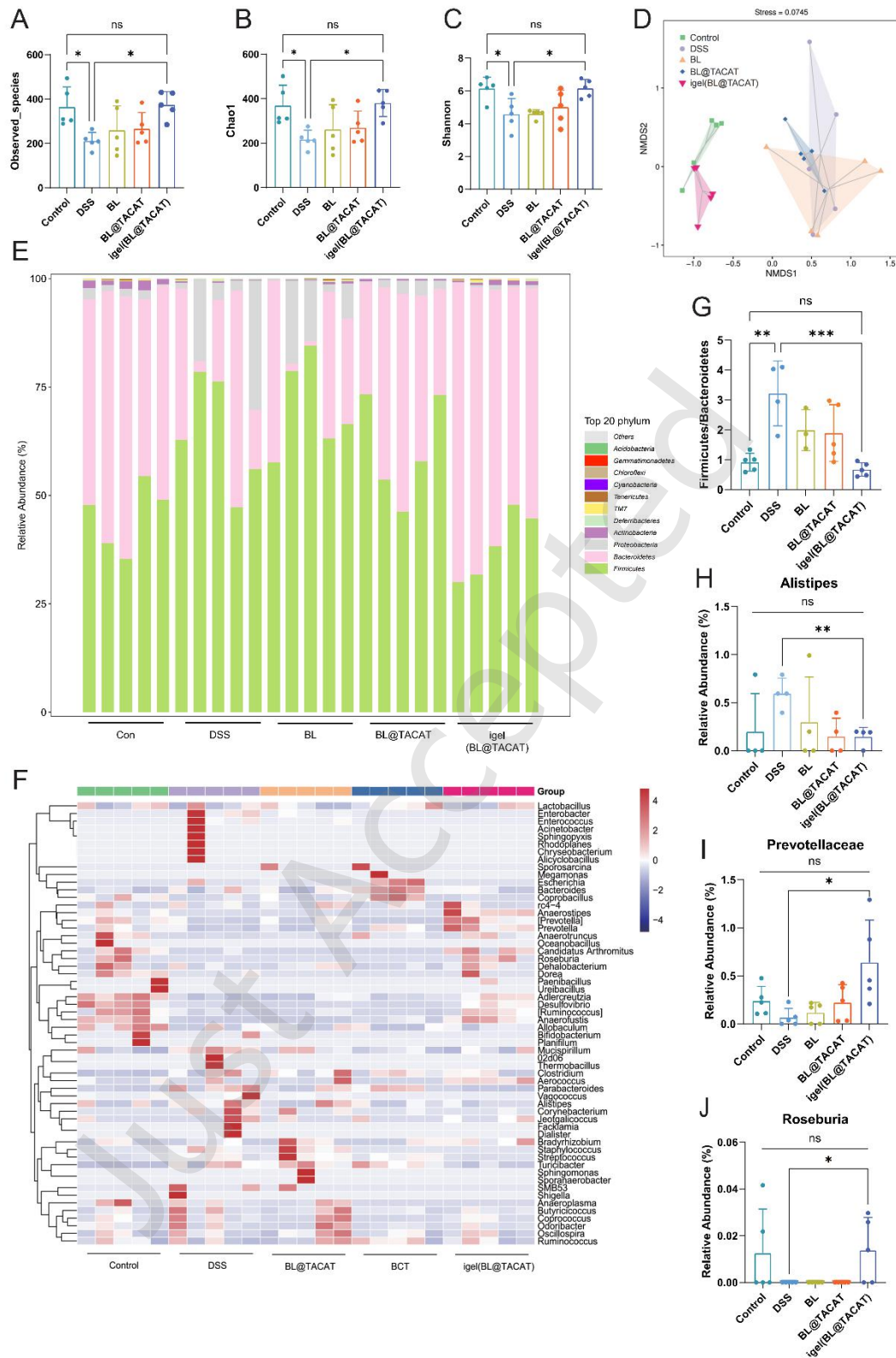


Figure 7 Igel(BL@TACAT) modulates gut microbiota composition. A-C) The alpha diversity index of observed species, Chao1, and Shannon. D) NMDS plot showing gut microbiome-diversity. E) Relative abundance of gut microbiota at the phylum level across treatment groups F) Heatmap illustrating relative abundances of gut microbiota at the genus level. G) Ratio of the phylum Firmicutes in Bacteroidetes. H-J) Relative abundances of selected taxa. Data are presented as the means $\pm$ SD (n = 4 or 5). Statistical analysis was performed using one-way ANOVA. *p < 0.05; ns, not significant

positive and F4/80-positive staining areas were reduced in mice treated with igel(BL@TACAT), suggesting that igel(BL@TACAT) could alleviate intestinal immune cell infiltration and inflammatory responses in the IBD mouse model (Fig. 5G). Quantitative fluorescence analysis of CD68 and

F4/80 also yielded consistent results (Fig. S7A, B). By regulating macrophage activity and distribution, igel(BL@TACAT) exhibited a favorable preventive effect.

3.6 Therapeutic Effect of igel(BL@TACAT) on DSS-Induced

Colitis

To systematically evaluate the therapeutic effect of igel(BL@TACAT) on inflammatory bowel disease *in vivo*, a mouse colitis model induced by 3% DSS was established. During the induction period of colitis with 3% DSS, all mice except those in the control group exhibited continuous body weight loss, indicating successful model establishment and progressive aggravation of inflammation (**Fig. 6A** and Fig S8). After different interventions were administered, it could be clearly observed that the igel(BL@TACAT) group showed a significantly smaller degree of body weight loss after treatment and recovered to a level close to that of the control group, whereas the recovery of body weight in the BL group and BL@TACAT group was not obvious and remained far below that of the igel(BL@TACAT) group (**Fig. 6B**). This indicated that in the absence of protection by inulin hydrogel microspheres, BL and BL@TACAT were susceptible to the effects of gastric acid, digestive enzymes, and other complex gastrointestinal environments during gastrointestinal transit, resulting in reduced survival rates. Similarly, the results of disease activity index (DAI) evaluation were consistent with the trend of body weight changes. Treatment with igel(BL@TACAT) decreased the DAI score in mice, whereas only limited improvement was observed in the BL and BL@TACAT groups (**Fig. 6C**), indicating that igel(BL@TACAT) exerted a therapeutic effect on colitis and alleviated colitis symptoms in mice. In addition, changes in the colons of mice in different groups could be visually observed through gross images (**Fig. 6D**). Compared with the DSS group, colon length in the igel(BL@TACAT) group was restored, and edema was alleviated, demonstrating a favorable repair effect. The conditions of the colons in the BL group and BL@TACAT group were also improved to some extent, although they had not recovered to the level of the healthy group (**Fig. 6E**). H&E staining further confirmed the efficacy of igel(BL@TACAT). In the igel(BL@TACAT)-treated group, morphological damage to the colon tissue was improved. Compared with the DSS group, the glandular arrangement of colon tissues in the igel(BL@TACAT)-treated group was more orderly, inflammatory cell infiltration was reduced, crypt morphology was normal, and intestinal structural integrity was restored (**Fig. 6F**). Myeloperoxidase (MPO) is a neutrophil-specific marker commonly used to evaluate neutrophil activation and migration during inflammatory responses. Therefore, MPO immunohistochemical staining was used to assess neutrophil infiltration in mouse colon tissues. The immunohistochemical staining results showed that igel(BL@TACAT) treatment suppressed DSS-induced upregulation of MPO expression, indicating that inflammatory responses were alleviated after treatment (**Fig. 6G** and Fig. S9).

Subsequently, the regulatory effects of catalase-modified probiotics encapsulated within inulin hydrogel microspheres on inflammatory cytokines in the IBD mouse model were evaluated. IL-1 β , IL-6, and TNF- α are typical pro-inflammatory cytokines that reflect immune activation and inflammatory responses, whereas IL-10 is an anti-inflammatory cytokine, and changes in its level help evaluate the inhibitory effect of treatment on intestinal inflammation. The results showed that, compared with DSS-treated mice, the concentrations of pro-inflammatory

cytokines, including IL-6, TNF- α , and IL-1 β , in the serum of mice in the igel(BL@TACAT) group were reduced (**Fig. 6H-J**). In contrast, the secretion level of the anti-inflammatory cytokine IL-10 was increased (**Fig. 6K**), indicating that treatment with igel(BL@TACAT) could suppress inflammatory responses in the mouse model of inflammatory bowel disease. Then, we further investigated the effect of igel(BL@TACAT) on intestinal barrier function. In IBD, destruction of the intestinal mucus layer is closely associated with inflammatory responses. AB/PAS staining is mainly used to detect goblet cells and mucins in colon tissues. Goblet cells and mucins were markedly reduced in DSS-induced IBD mice, whereas the mucus layer was improved in the colon tissues of mice treated with igel(BL@TACAT), with well-preserved goblet cell structures and increased mucin secretion (**Fig. 6L**). In addition, IBD is usually accompanied by intestinal barrier damage and loss of tight junctions, resulting in increased intestinal permeability. ZO-1 and Occludin are key components of intestinal epithelial tight junctions and play important roles in maintaining intestinal barrier integrity and regulating epithelial permeability^[46, 47]. Furthermore, Immunofluorescence images of ZO-1 and Occludin showed that the expression levels of ZO-1 and Occludin were decreased in DSS-treated mice. However, the restored expression levels of ZO-1 and Occludin were observed in mice treated with igel(BL@TACAT) (**Fig. 6M**). Quantitative fluorescence analysis of ZO-1 and Occludin-1 also yielded consistent results (Fig. S10A, B).

3.7 Regulatory Effect of igel(BL@TACAT) on the Gut Microbiota

Existing evidence has shown that gut microbiota dysbiosis, characterized by reduced microbial diversity and depletion of beneficial bacteria, is closely associated with inflammatory bowel disease (IBD) and many other diseases. In recent years, probiotic therapy has demonstrated promising potential in the treatment of IBD because of its ability to regulate the intestinal microecological environment. In this study, 16S rRNA gene sequencing was performed to analyze changes in the colonic microbiota of mice and evaluate the regulatory effect of catalase-modified probiotics encapsulated within inulin hydrogel microspheres on the colonic microbiota of IBD mice.

Through microbial diversity analysis, the diversity of intestinal microbiota in different groups of mice was evaluated. As shown in Fig. S11, the rarefaction curves for all five sample groups tended to be stable, indicating that the sequencing depth was sufficient to capture the most of microbial diversity. The α -diversity analyses, including Observed OTUs, Shannon index, and Chao1 index, were used to assess differences in microbial community structures. Compared with the DSS group, the OTU richness (**Fig. 7A**), Chao1 index (**Fig. 7B**), and Shannon index (**Fig. 7C**) in the igel(BL@TACAT)-treated group were all increased, indicating enhanced microbial diversity. β -diversity analysis using NMDS plots showed a distinct clustering of intestinal microbiota composition in the DSS-treated group (**Fig. 7D**). Notably, the bacterial community structure in the igel(BL@TACAT) group closely resembled that of the control group, indicating that it effectively restored the intestinal microbiota.

Regarding bacterial community abundance, detailed taxonomic analyses were performed at both the phylum and genus levels. At the phylum level, compared with the control group, the

abundance of Firmicutes increased and the abundance of Bacteroidetes decreased in the DSS group (Fig. 7E). These compositional changes were significantly reversed after treatment with igel(BL@TACAT). Subsequently, the Firmicutes-to-Bacteroidetes ratio (F/B ratio) was evaluated. Firmicutes and Bacteroidetes account for 80–90% of the intestinal microbiota, and the F/B ratio is often considered one of the indicators of intestinal microecological imbalance. Patients with IBD, obesity, and other conditions often exhibit a higher Firmicutes/Bacteroidetes ratio. Such changes may indicate overgrowth of harmful bacterial populations, disruption of microbial homeostasis, or deterioration of intestinal health. The results showed that, compared with the control group, the F/B ratio was markedly elevated in the DSS group, whereas treatment with igel(BL@TACAT) reduced the F/B ratio to a level close to that of healthy mice, confirming the effectiveness of igel(BL@TACAT) in alleviating DSS-induced microbiota dysbiosis (Fig. 7G). At the genus level (Fig. 7F), the relative abundance of *Alistipes*, which is associated with inflammatory responses and colorectal cancer, was reduced (Fig. 7H), whereas the abundances of beneficial bacteria such as *Prevotellaceae* (Fig. 7I) and *Roseburia* (Fig. 7J) were increased. We further applied the LEfSe algorithm to identify differentially abundant taxa among the three groups. In the DSS group, the expression levels of 13 taxa, including Lachnospiraceae ruminococcus and Erysipelotrichaceae clostridium, were decreased compared with those in the control group, while the expression levels of 7 taxa, such as Deferribacteraceae mucispirillum and Staphylococcaceae staphylococcus, were overexpressed. (Fig. S12A, B). In the igel(BL@TACAT) group, the expression levels of 8 taxa (Deferribacteraceae mucispirillum and Staphylococcaceae staphylococcus) were lower compared with those in the DSS group, whereas the 6 taxa (including Lactobacillaceae lactobacillus and Desulfovibrionaceae desulfovibrio) were expressed at higher levels (Fig. S12C, D). Collectively, these results indicated that BL@TACAT encapsulated within inulin hydrogel microspheres improved colitis by positively regulating the gut microbiota and ultimately restoring microbial homeostasis.

4 Conclusions

In summary, to address the limitations of current therapeutic strategies for inflammatory bowel disease (IBD), which struggle to simultaneously achieve anti-inflammatory effects, antioxidant activity, and gut microbiota reconstruction, we developed an oral delivery system that integrates multifunctionally engineered probiotics with a degradable polysaccharide hydrogel, and systematically evaluated its *in vitro* and *in vivo* functionality and safety.

We selected *Bacillus licheniformis* as the target probiotic. A stable coating layer was constructed on its surface via a metal–phenolic network formed by tannic acid and ferric ions, and catalase was subsequently immobilized onto this metal–phenolic network, successfully generating an antioxidant-functional BL@TACAT system. To further enhance the gastrointestinal stability and intestinal colonization capacity of engineered probiotics, inulin was modified with methacrylate groups in this study, and catalase-loaded *Bacillus licheniformis* (BL@TACAT) was encapsulated into hydrogel microspheres through photo-initiated emulsion polymerization.

In vitro studies demonstrated that igel(BL@TACAT) exhibited antioxidant activity and good biocompatibility. In a DSS-induced IBD mouse model, both prophylactic and therapeutic administration of igel(BL@TACAT) alleviated disease symptoms, reduced disease activity index, restored body weight, and repaired colonic architecture. Meanwhile, it effectively protected the intestinal barrier by regulating the secretion of inflammatory cytokines. 16S rRNA sequencing revealed a significant recovery of gut microbiota diversity, with an increase in beneficial bacteria and a decrease in pathogenic bacteria, indicating restoration of microbial homeostasis.

Overall, this study successfully established a novel oral therapeutic strategy integrating antioxidant activity, probiotic therapy, and targeted delivery, providing new insights and experimental evidence for the comprehensive treatment of IBD.

Electronic Supplementary Material: Supplementary material (please provide the brief detail of the ESM) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94909228>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.”

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Declaration of competing interest

All the contributing author report no conflict of interests in this work.

Author contribution statement

“Chen Kan: Data curation, writing manuscript, experimental design. Qiangyuan Fan: Data curation, experimental design. Zijiu Sun: Data curation, experimental design. Huanhuan Zhu: Data curation. Yue Zhang: Data curation. Kesheng Wang: Data curation. Lin Han: Data curation. Shaobo Xue: experimental design. Hui Ren: experimental design. Fenyong Sun: project administration, funding acquisition. Yanqiang Hou: project administration, validation experimental design. Zunzhen Ming: project administration, experimental design. Shiyu Sun: experimental design, project administration, funding acquisition. All the authors have approved the final manuscript.”

CRedit author statement (<https://credit.niso.org/>) is recommended to use, and all the listed authors should meet the definition of Author in ICMJE recommendations (<https://www.icmje.org/>).

Informed consent

Not applicable.

Ethics statement

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals issued by the

National Institutes of Health (NIH) and were approved by the Animal Ethics Committee of Shanghai Tenth People's Hospital (Ethical code: SHDSYY-2025-6129).

Use of AI statement

"None."

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Electronic Supplementary Material

Inulin hydrogel microspheres delivering catalase-modified probiotics for inflammatory bowel disease therapy

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Fig S1. Suspension and deposition characteristics of native BL and BL@TACAT. The suspensions of BL@TACAT turned black purple

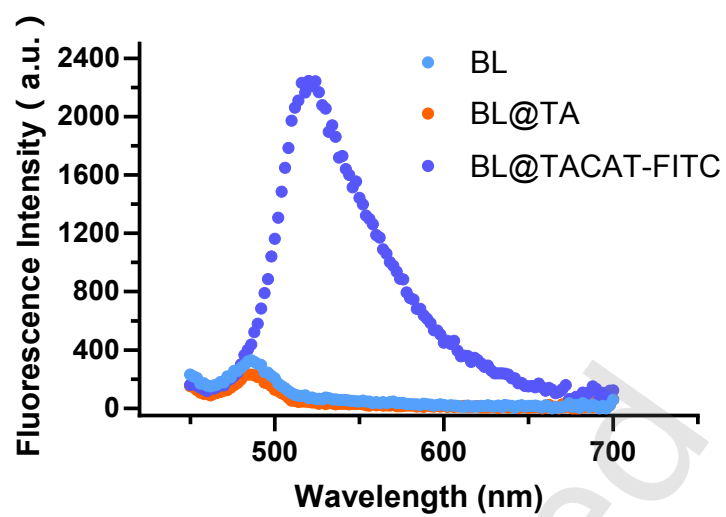


Fig S2. Fluorescence intensity was determined using a microplate reader

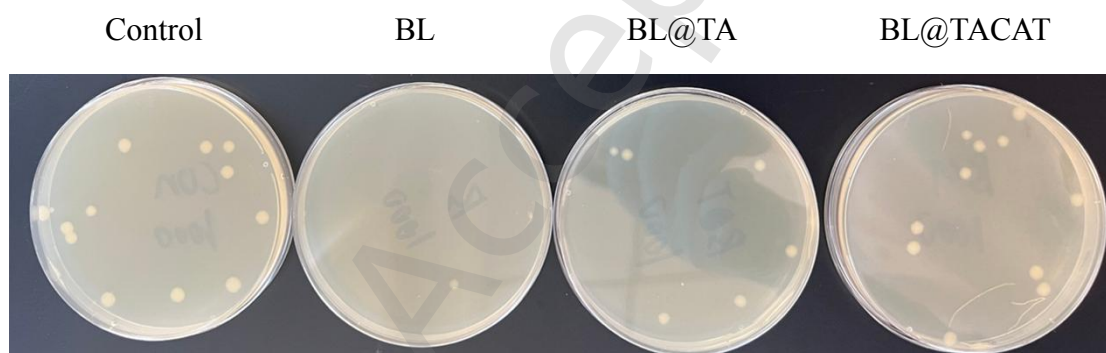


Fig S3. Plate coating photos of each group after incubation with H_2O_2

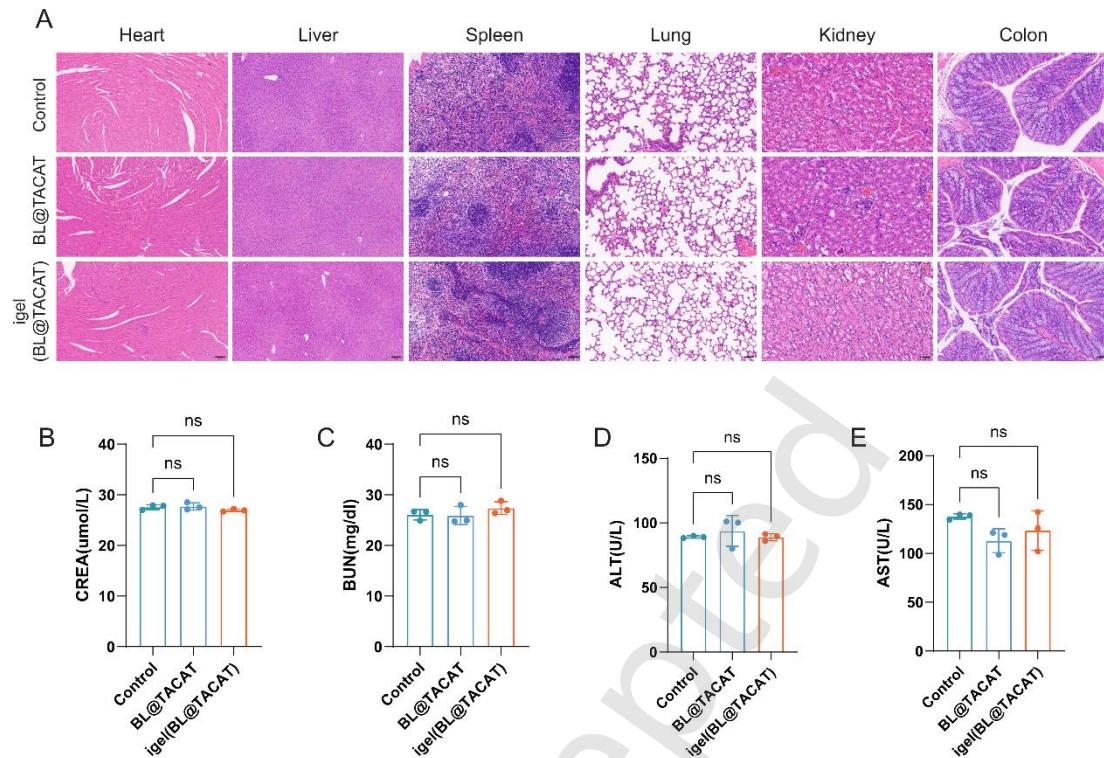


Fig S4 The biosafety of igel(BL@TACAT). A) Histological examination of the major organs. Scale bar: 50 μm . B-E) Serum analysis of CREA, BUN, ALT, AST in mice after different treatments. (n=3).

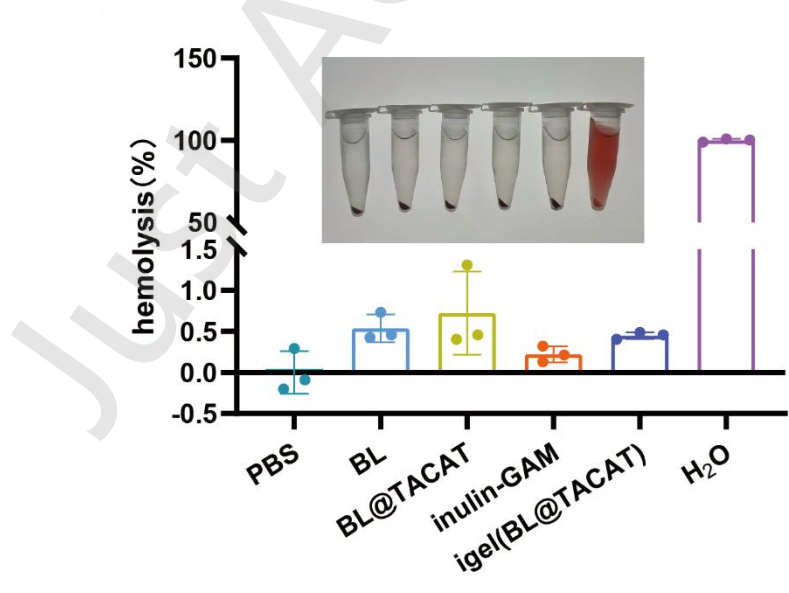


Fig S5. Hemolytic rate of different treatment, and H₂O; Inset: digital photograph of the hemolytic test.

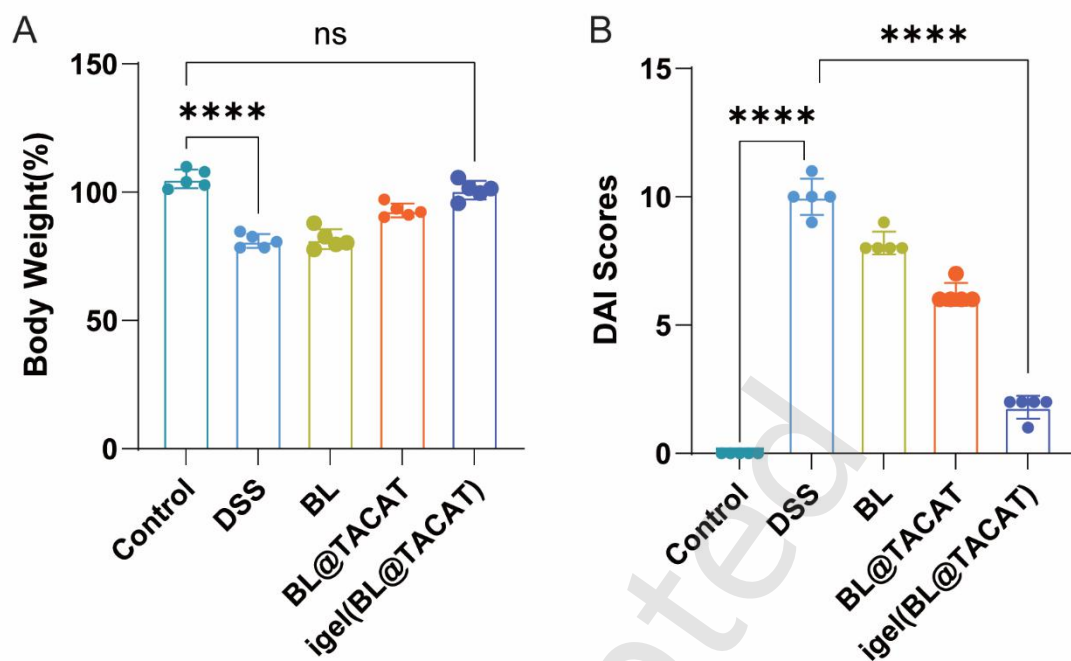


Fig S6. The Body weight (A) and DAI (B) Scores of mice after prophylactic modeling completion

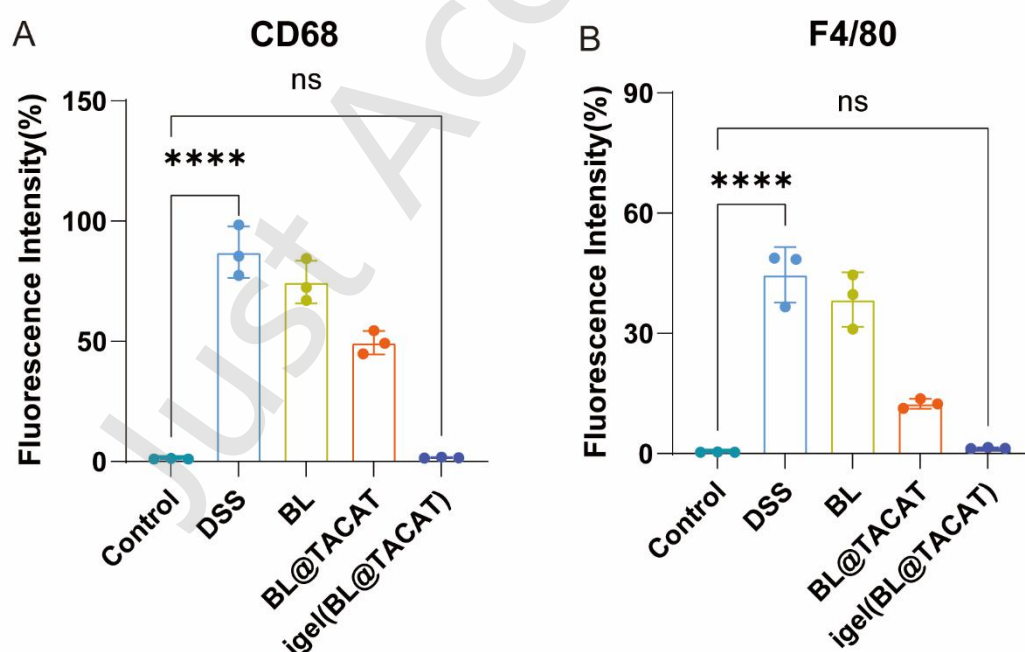


Fig S7. Quantitative fluorescence analysis of CD68 (A) and F4/80 (B).

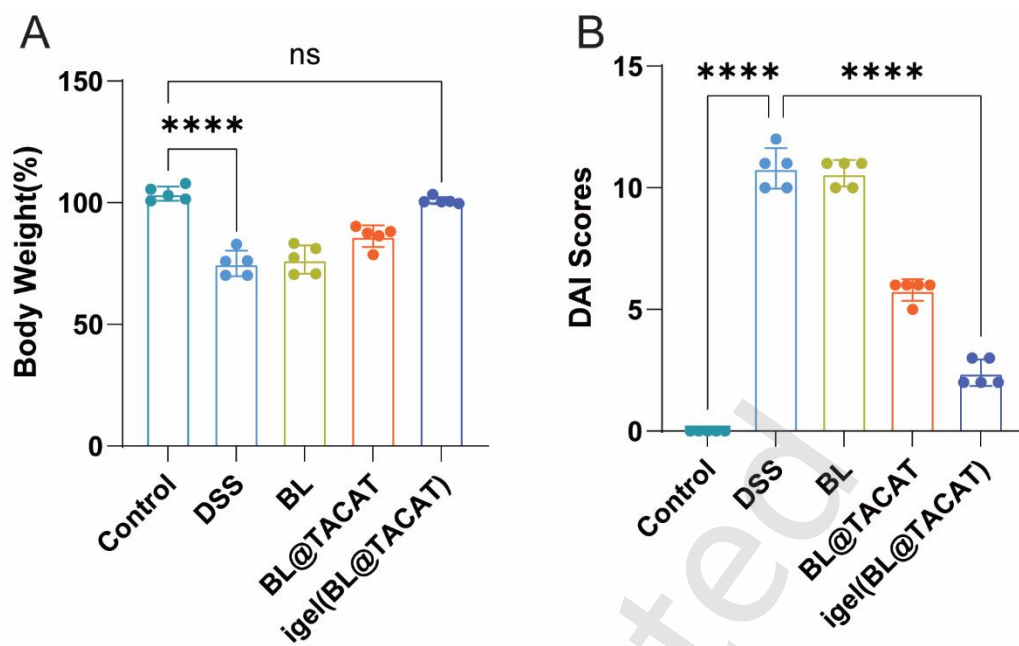


Fig S8. The Body weight (A) and DAI Scores (B) of mice after therapeutic modeling completion

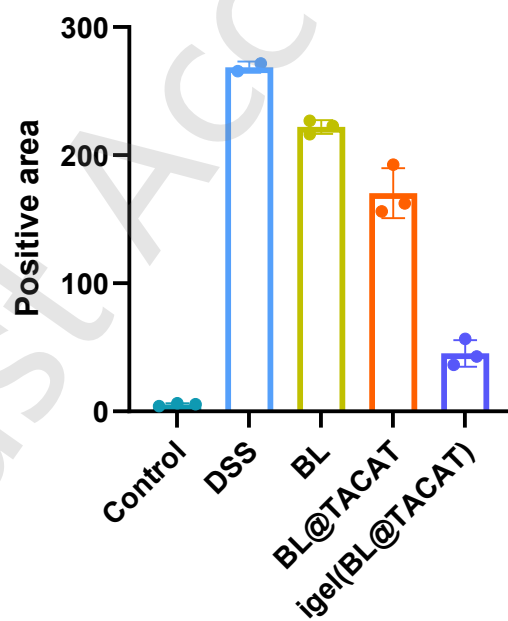


Fig S9. Quantitative analysis of MPO immunohistochemical staining in colon sections.

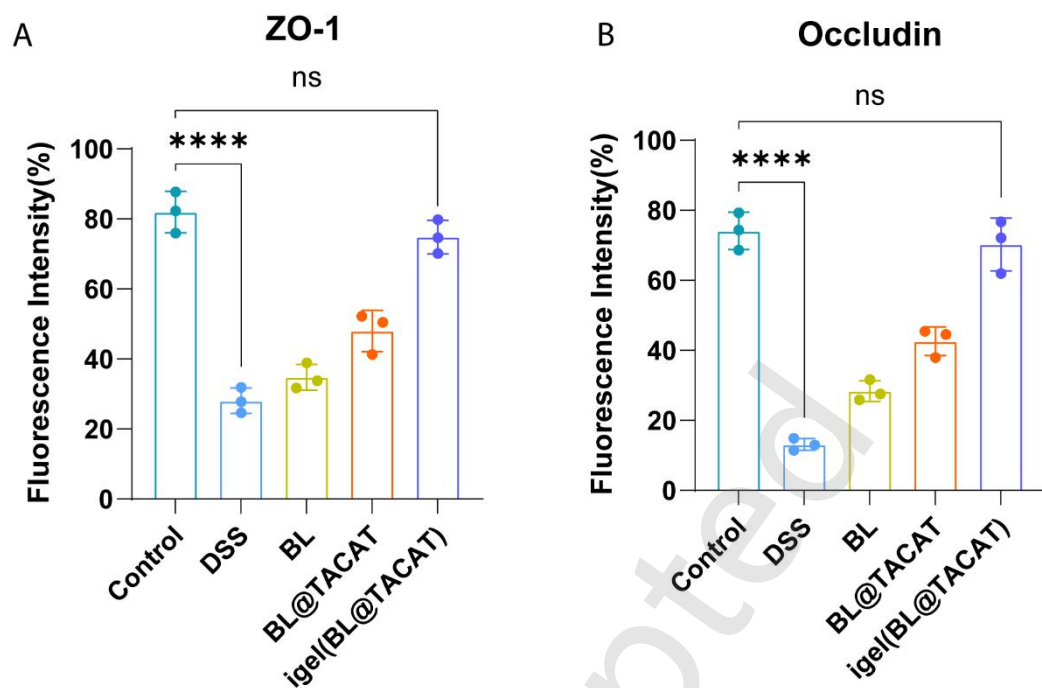


Fig S10. Quantitative fluorescence analysis of ZO-1 (A) and Occludin (B).

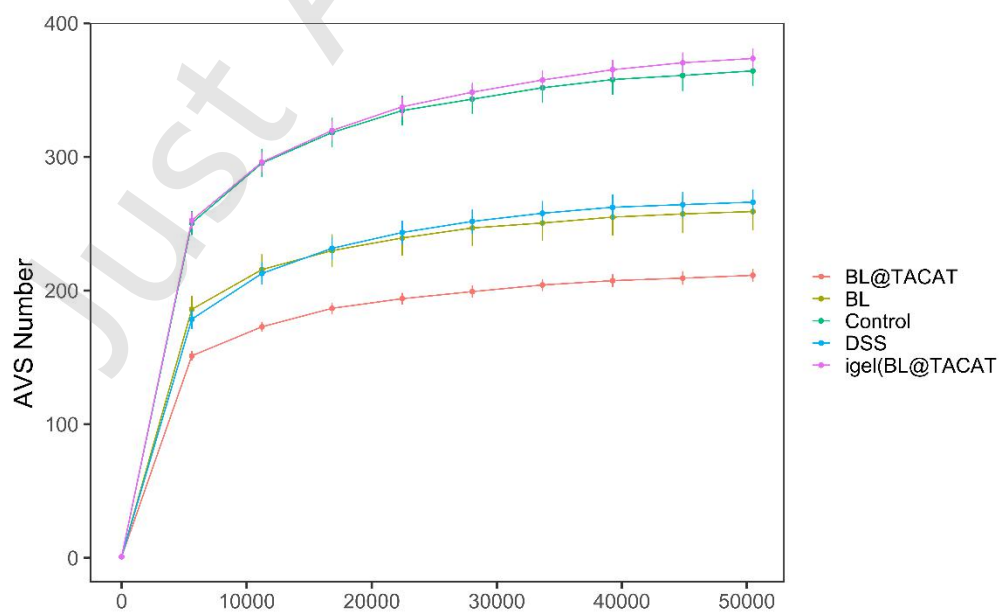


Fig S11. Rarefaction curve.

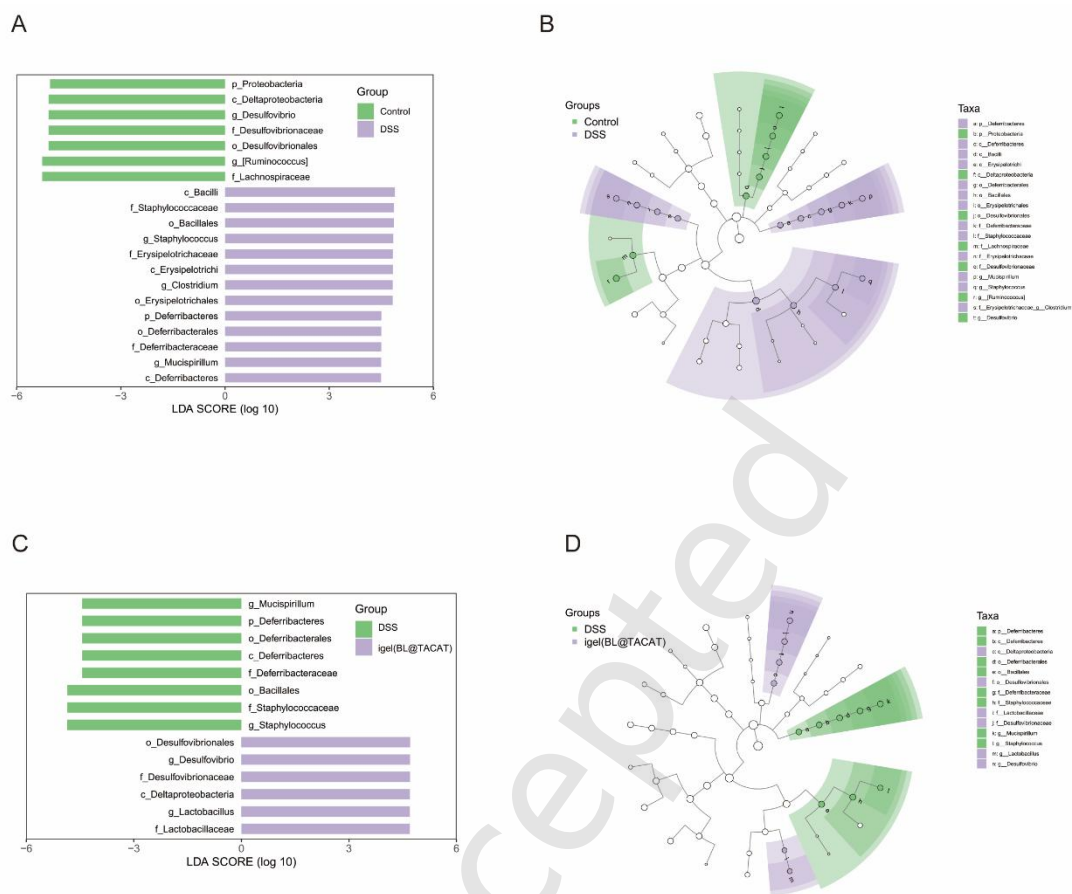


Fig S12. A, B) Linear discriminant analysis Effect Size (LEfSe) analysis of the microbiota of Control and DSS. C, D) Linear discriminant analysis Effect Size (LEfSe) analysis of the microbiota of DSS, igel(BL@TACAT).