Supporting Information

Intestinal models based on biomimetic scaffolds with ECM micro-architecture and intestine macro-elasticity: close to intestine tissue and immune response analysis

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1. Materials

Gelatin was purchased from Sinopharm Group Chemical Reagent (Shanghai, China). Bacterial cellulose (BC) was purchased from Hainan Yide Co., LTD. Phosphate buffered saline (PBS) was purchased from Genom Biomedical Technology Co., LTD. 4% Paraformaldehyde (BL539A) was purchased from Beijing Lanjieke Technology Co., LTD. Hexafluoroisopropanol, TEMPO, 2-(*N*-Morpholino) ethane sulfonic acid (MES), NaBr, methyl thiazolyl tetrazolium (MTT), dimethyl sulfoxide (DMSO), glutaric dialdehyde, GlutaMAX Supplement, Y-27632, N-Acetyl-L-cysteine, EDTA, bovine serum albumin (BSA), nicotinamide, SB202190, A83-01, and Triton X-100 Type IV collagenase were purchased from Sigma-Aldrich (Shanghai, China). Fetal bovine serum (FBS), Advanced DMEM/F-12, 1 M HEPES, 100×N-2 supplement, 50×B-27 supplement, and 100×antibiotic-antimycotic were purchased from Thermo Fisher Scientific (Shanghai, China). Propidium iodide, Calcein-AM, alkaline phosphatase assay kit, and annexin V-FITC apoptosis detection kit were purchased from Shanghai Beyotime Biotechnology Co., LTD. Mouse Interleukin-10 ELISA kit and mouse tumor necrosis factor-α ELISA kit were purchased from Wuhan Baishi Biological Engineering Co., LTD. Mucin 2 antibody, sucrase-isomaltase antibody, and chromogranin-A antibody were purchased from Santa Cruz Biotechnology, Inc. Anti-ZO1 tight junction antibody, anti-lysozyme antibody, anti-LGR5 antibody, anti-Villin antibody, donkey anti-rabbit 488, donkey anti-mouse 555 were purchased from Abcam Trading Co., LTD (Shanghai, China). Transwell inserts (24-well dish type, 0.4 μm pore size, 6.5 mm diameter, and 0.33 cm^2 area, no. 3413) with polycarbonate membranes, 100 μm cell filter, 96-well cell culture plate, 24-well cell culture plate, 15 mL centrifuge tube, 50 mL centrifuge tube were purchased from Corning Inc. Millipore 0.22 μm sterile needle filter was purchased from Millipore Trading Co., LTD (Shanghai, China). Caco-2 cell was provided by the Cell Library of the Chinese Academy of Sciences (Shanghai, China). *L. reuteri* CICC 6119, *L. casei* CICC 6104， and *B. infantis* CICC 21716 were obtained from the China Center of Industrial Culture Collection. *E. coli* ATCC 25922 was obtained from the National Center for Medical Culture Collections (Beijing, China). Male C57BL/6 mice aged 6-8 weeks were purchased from Hangzhou

Ziyuan Experimental Animal Science and Technology Co., LTD.

2. RT-qPCR assay

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is used to measure the RNA transcription levels of cells. TRIzol is used as the extraction reagent. cDNA is synthesized using TRI Reagent by the manufacturer's protocol. The RNA samples are treated with RQ1 RNase-free DNase to digest any residual chromosomal DNA and then quantified using a UV spectrophotometer. For first-strand cDNA synthesis, initially 5 μL of a reaction containing 2 μg of total RNA, 1 μL of 10 mM dNTP mix, and 1 μL of 100 μM oligo dT primer are incubated at 65 °C for 10 min, spun briefly, and placed in ice. To this, 2 μL of 5× M-MuLV reverse transcriptase buffer, 0.25 μL of 20 U/μL M-MuLV Reverse transcriptases, and 0.25 μL of Ribolock RNase Inhibitor are added and made up to 10 μL with sterile nuclease-free water. The reaction mixture is incubated at 37 °C for 60 min, then heated to 95 °C for 10 min, and then stored at − 20 °C until use. Later, 2.0 μL of the cDNA, along with SYBR Premix Ex Taq and 0.25 μM of individual primer sets (Table S1) are added to a 10 μL reaction subjected to thermo-cycling following standard protocols. Gene relative expressions are calculated according to the method of $2^{-\Delta\Delta CT}$, and the values are normalized to the housekeeping gene GAPDH.

Table S1. Primers of RT-qPCR

	Forward	Reverse
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
ZO-1	GTTGATCTGAAGTGATAGGTGGA	CACTATGAAACAGACTACACGACA
Villin	CGGAAAGCACCCGTATGGAG	CGTCCACCACGCCTACATAG
SI	TCCAGCTACTACTCGTGTGAC	CCCTCTGTTGGGAATTGTTCTG
Chg-A	ACTCCGAGGAGATGAACGGA	CTTGGAGAGCGAGGTCTTGG
Lysozyme	CGCTACTGGTGTAATGATGG	TTTGCACAAGCTACAGCATC
Muc-2	TGCCTGGCCCTGTCTTTG	CAGCTCCAGCATGAGTGC
Lgr-5	GAGAAAGCATTTGTAGGCAAC	ATCTCCCAACAAACTGGATG

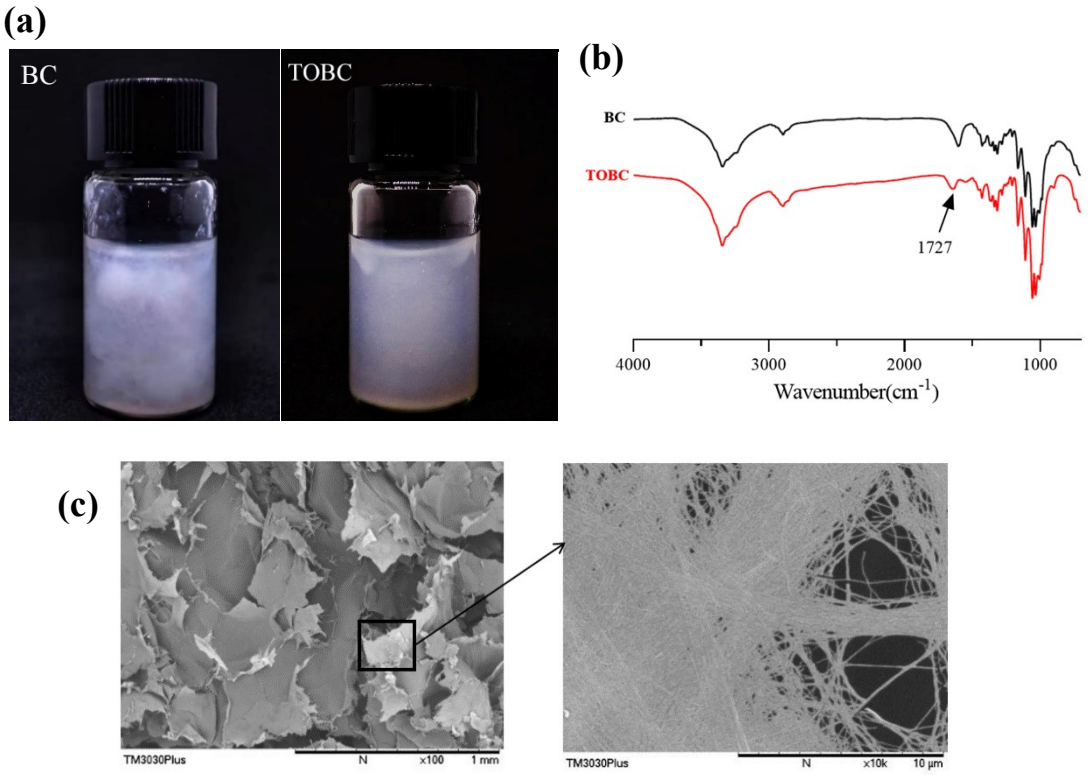

Figure S1. (a) Pictures of BC and TOBC in water; (b) ATR-FTIR spectra of BC and TOBC; (C) SEM pictures of lyophilized TOBC.

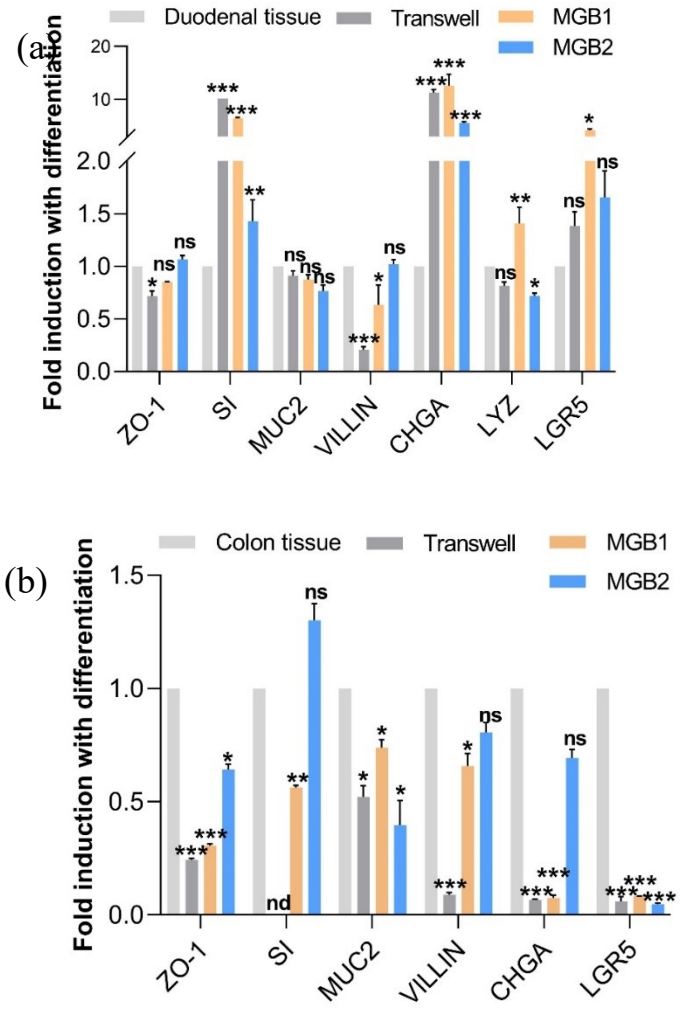

Figure S2. RT-qPCR gene expression analysis of (a) duodenal epithelium cells and (b) colon epithelium cells cultured on scaffolds, fresh duodenal tissues and colon tissues are used as a positive control (light gray bars) and data are presented as fold change relative to the fresh duodenal tissues (nd, not detected; ns, not significant).