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Supplemental Information

Bioinspired Anti-digestive Hydrogels Selected by a Simulated Gut Microfluidic Chip for Closing Gastrointestinal Fistula

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Supplemental information:

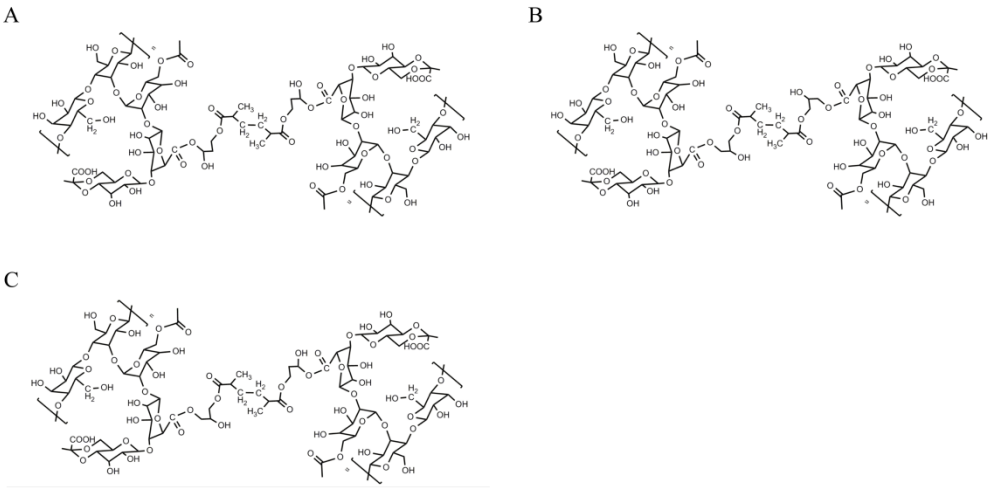

Figure S1, related to Figure 1A. (A-C) Three combinations of photopolymerized xan-GMA due to the presence of its isomers.

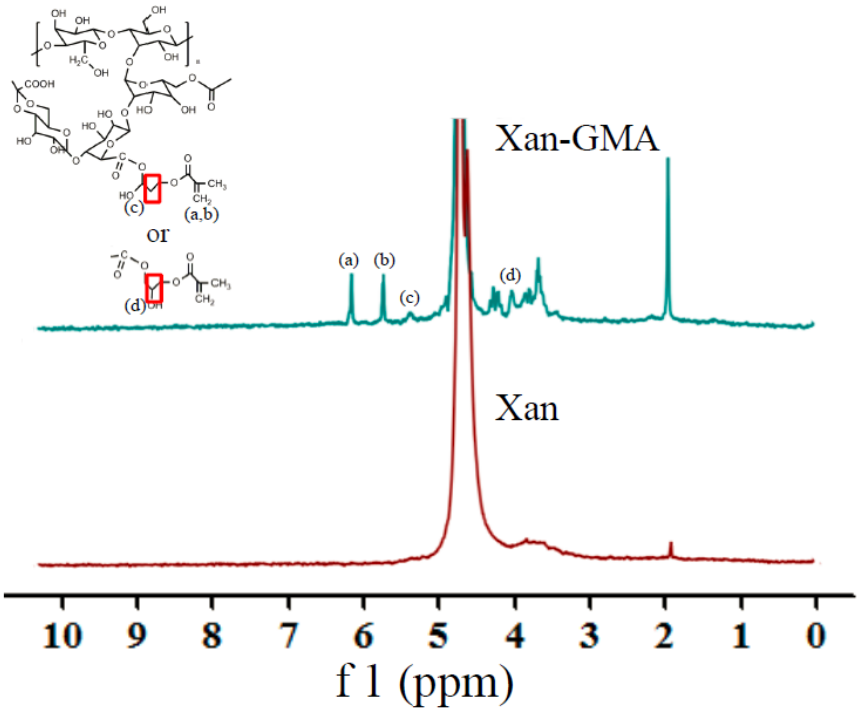

Figure S2, related to Figure 1B. The magnified version of Figure 1B regarding the ^{1}H-NMR spectrum, a=6.17, b=5.76, c=5.06 and d=4.02.

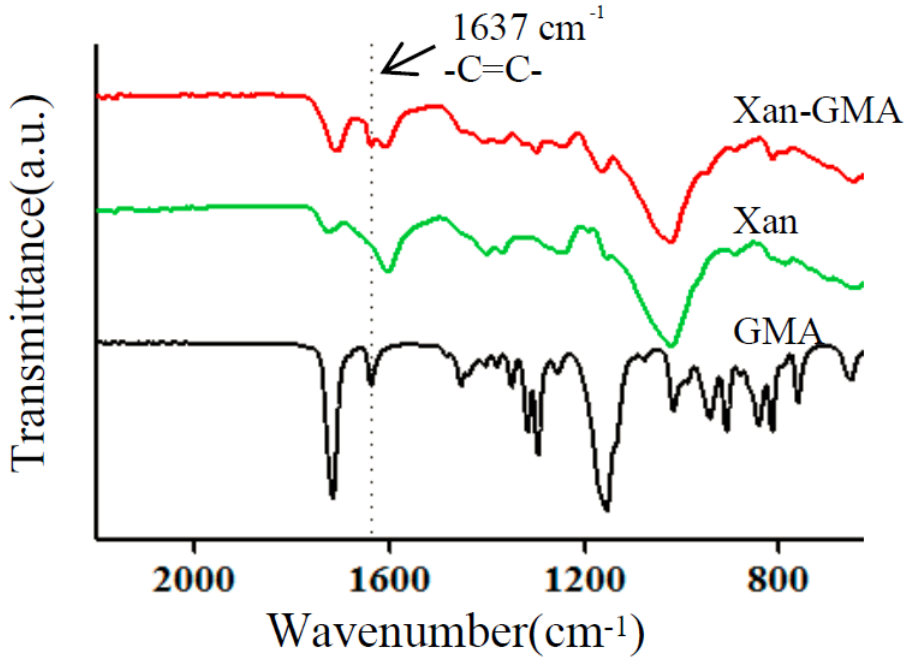

Figure S3, related to Figure 1C. The magnified version of Figure 1C regarding the FTIR spectrum, the peak at 1637cm^{-1} refers to the C=C groups in GMA.

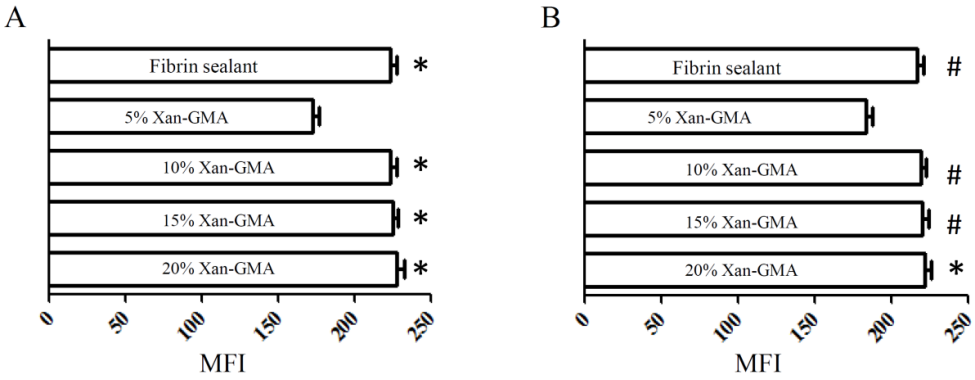

Figure S4, related to Figure 3A. Semiquantitative fluorescence intensity of TJ proteins using Image J software. (A) Occludin-1, compared with 5% xan-GMA, $^{*}P$<0.001. (B) ZO-1, compared with 5% xan-GMA, $^{\#}P$<0.01, $^{*}P$<0.001, MFI: mean fluorescence intensity.

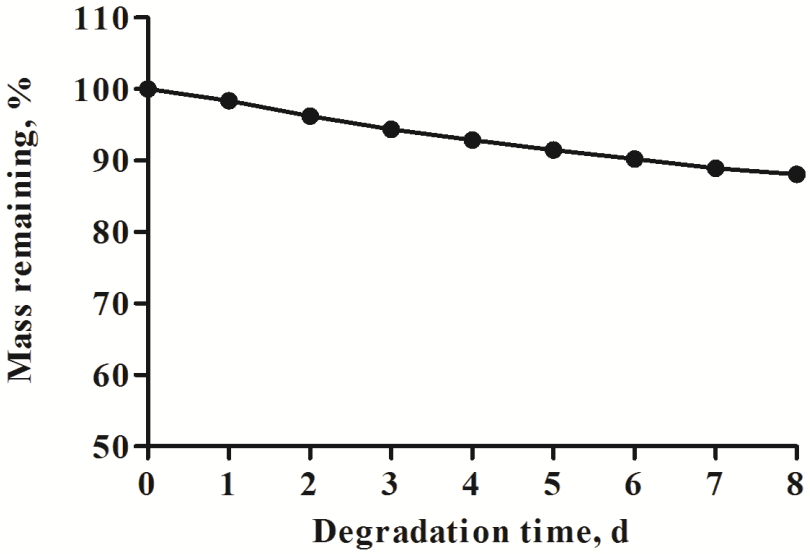

Figure S5, related to Figure 6. The degradation behavior of the 10% xan-GMA hydrogels in the presence of IEC-6 cells, indicating the xan-GMA hydrogels would become a hindrance at the late stage of fistula repair if not removed.

Transparent Methods:

1. Materials

Xanthan gum (viscosity of 1% aqueous solution at 20°C: 1450-2000 mPa.s, TCI Development Co. Ltd, Shanghai, China); GMA (Aladdin®, Shanghai, China); hydrochloric acid (36-38wt%, Jiuyi Reagent Co. Ltd, Shanghai, China); 2-hydroxy-4'-(2-hydroxyethoxy) -2-methylpropiophenone (I-2959, Sigma-Aldrich®, St. Louis, MO, USA); polydimethylsiloxane (PDMS, generated from the liquid silicon elastomers A and B liquid mixing at a volume ratio of 10 : 1, silicon elastomer was purchased from Dow Corning Corp, Midland, Michigan, USA); uncontaminated intestinal juice (obtained from a patient with duodenal fistula, amylase was quantified as 20000 U/L using a dry chemistry method in the laboratory department of Jinling Hospital). All other reagents were of analytical reagent grade.

2. Synthesis of xan-GMA

The protocol of xan-GMA synthesis was first established. First, 0.6% (w/v) xanthan aqueous solution was prepared by dissolving xanthan powders into deionized water, followed by stirring overnight. Then, 270 mL of such solution was poured into a round-bottom flask and heated to 80 °C using an oil bath (model: DF-101S, Xinbao Instrument Co. Ltd, Dongguan, China). Afterwards, hydrochloric acid was used to adjust its pH to 4.2-4.8, and 1.88 mL of GMA was added dropwise. After 12 h of reaction at 80 °C, the resultant solution was collected and then dialyzed for 3 d using dialysis membranes (molecular weight cut-off: 12-14 kDa) to remove unreacted residues. Finally, the solution was lyophilized in a freeze dryer (Xiongdi Instrument Co. Ltd, Zhengzhou, China) and stored in a sealed bag at 4 °C.

3. Synthesis of xan-GMA hydrogels

Xan-GMA was dissolved in deionized water containing 0.1% (w/v) I-2959 to obtain solutions with different concentrations at 5% (w/v), 10% (w/v), 15% (w/v) and 20% (w/v). Gelation of xan-GMA solution was initiated under UV-light exposure (365 nm, ~6.0 W/cm^2, model: UVPL-411, Yunhe Tech Co. Ltd, Suzhou, China) for 40s.

4. Confirmation of GMA conjugation to xanthan

4.1 Fourier-transform infrared (FTIR) spectrometry. An FTIR spectrophotometer was

applied to investigate the FTIR spectra of GMA, xanthan and xan-GMA using a Nicolet-6700 spectrometer (Thermo®, USA) at room temperature, in the wave number range of 4000–500 cm^{-1} using the KBr pellet technique. The liquids or powders were ground to a dry KBr disk and 32 scans at a resolution of 4 cm^{-1} were used to record the spectra.

4.2 NMR spectrum detection. Xanthan and xan-GMA were dissolved in deuterated solvents. The modification of xanthan and the purity of xan-GMA in final products were characterized by ^{1}H NMR (Bruker 500, Germany).

5. Morphology

The porosity of hydrogels that mimics the extracellular matrix can support cell growth and tissue regeneration. This structure was observed under a scanning electron microscope (SEM; model: S-4800, Hitachi®, Japan). Specifically, the xan-GMA hydrogels with varied xan-GMA concentrations were freeze-dried and their vertical sections were imaged after coating with a thin layer of gold.

6. Swelling ratio

The swelling ratio of xan-GMA hydrogels was determined by the following formula (Ghobril and Grinstaff, 2015):

$$\text{Swelling ratio} = (M_{\text{swollen gel}} - M_{\text{dried gel}}) / M_{\text{dried gel}}$$

$M_{\text{dried gel}}$ and $M_{\text{swollen gel}}$ stand for the mass of dried hydrogel and swollen hydrogel at equilibrium, respectively. Specifically, tested hydrogels were immersed in phosphate-buffered saline (PBS). At each preset time, they were removed, and the surface moisture was immediately wiped off using tissue paper. After the hydrogels were weighed, they were returned to the PBS buffer. This process was repeated until equilibrium was attained. To confirm that the swelling ratio was changed with xan-GMA concentration, the correlation of swelling ratio and pore density per visual field at 50× magnification was described using Graphpad Prism 5 software.

7. Rheometry

Rheological properties of the hydrogels were characterized using a rheometer (model: MCR302, Anton Paar Co. Ltd, Austria) with the parallel plates at a gap size of 1mm. In the oscillatory strain sweep experiment, the constant frequency was fixed at 10 Hz,

while in the oscillatory frequency sweep experiment, the constant strain was fixed at 1%. For the oscillatory time sweep experiment, an external UV-light with three on-and-off cycles was applied to the hydrogels with the constant strain at 1% and frequency at 10 Hz.

8. Injectability

This injectability of xan-GMA hydrogels was also tested using the rheometer. The steady state shear flow was from 0.1 to 500s^{-1} of shear rate. Additionally, the practice of injection was performed on the 10% xan-GMA solution by handwriting the logo of Southeast University, SEU. To make the logo clearer, the dye of rhodamine 123 (Aladdin®, Shanghai, China) was added to the solution.

9. Swelling-shrinking property

The role of $CaCl_2$ on the shrinking of xan-GMA hydrogels was investigated as follows. First, tested hydrogels were immersed in deionized water and weighed at each preset time until the weight tended to be stable. Later, they were moved into 0.2mol/L $CaCl_2$ solution and weighed at each preset time until the weight became stable. The entire process was repeated three times. In addition, the concentration of $CaCl_2$ solution was changed to 0.5 mol/L or 0.8 mol/L to study the influence of the concentration on the shrinking ability of the hydrogels. Moreover, the conformation of the hydrogels in deionized water or 0.2 mol/L $CaCl_2$ solution was studied using the XRD assay (model: XD-3A, Shimadzu®, Japan).

10. Anti-digestive property

The anti-digestive performance was detected using a simulated gut microfludic chip. This chip was tailor-made through a series of processes including model code programming, 3D wax jetting, PDMS replicating, wax dissolving and PDMS-to-glass bonding (Li et al., 2017). The chip could mimic the anatomy of GI fistula. At the "fistula sites", we examined the anti-digestive property of the tested gels. Specifically, the fibrin sealant and the xan-GMA hydrogels were gelated in glass capillaries (~100 μm in diameter, Changcheng Instrument Co. Ltd, Shanghai, China), followed by insertion at the "fistula sites". The intestinal juice was pumped at 37°C at 0.2mL/h using a syringe pump (model: LSP04-1A, LongerPump®, China). The anti-digestive

property was evaluated by the ratio of digested part to the full-length.

11. Maintenance of gut barrier functions

The functions of IEC-6 cells (KeyGEN®, Nanjing, China) were studied by cell culture on the hydrogel surface. Specifically, the fibrin sealant and xan-GMA hydrogels were injected at the bottom of confocal dishes (thickness: ~1mm) and gelated by UV-light exposure. After washing twice using PBS, 1 mL of 5×10^5/mL IEC-6 cells were added in the dishes and cultured in a cell incubator. Three days later, IEC-6 cells were fixed with 4% (w/v) paraformaldehyde for 30 min at room temperature, and then proceeded to 0.05% (w/v) Triton X-100 for permeabilization. For immunofluorescent analysis of tight junction (TJ) proteins, we labeled the cells with ZO-1 antibody conjugated with FITC (green) (ab150266, Abcam®, USA) diluted at 1/20 and occludin antibody (sc-8145, Santa®, USA) using 1/100 dilution, followed by the donkey anti-goat IgG (ab150130, Alexa Fluor555®, USA) secondary antibody at 1/1000 dilution (red). The nuclear counter stain was DAPI (blue). A confocal scanning microscope (model: FV1000, Olympus®, Tokyo, Japan) was used for image analysis. Moreover, proliferation of IEC-6 cells on the hydrogel surface was detected using MTT assay. Similarly, the fibrin sealant and xan-GMA hydrogels were gelated at the bottom of a 96-well plate (thickness: ~1mm). 100μL of 2×10^4/mL IEC-6 cells were added to the wells, followed by adding 100μL Dulbecco's modified Eagle's medium. After incubation for 72 h, 20 μL of MTT (5 mg/mL) was added to each well and further incubated for 4 h. Formazan salt was dissolved in 200μL DMSO. After it was thoroughly dissolved, the resultant solution was transferred to another 96-well plate and measured with a microplate spectrophotometer (BioTek Instrument Co. Ltd, USA) at 570 nm. Each sample was replicated in three wells.

12. Degradation behavior of the hydrogel in the presence of IEC-6 cells

The 10% xan-GMA hydrogels in a 24-well plate were weighed at equilibrium (Wi), where the weight of the swollen hydrogels in the cell culture media of DMEM (KeyGEN®, Nanjing, China) is steady. Then, 1 mL of 5×10^5/mL IEC-6 cells were added in each well. At different time points, we removed the media of the specific wells and weighed the hydrogels (Wd). Meanwhile, we also re-introduced the fresh

media to the rest of the wells. The weight remaining percentage (Wr) of the hydrogel was calculated as follows (Ghobril and Grinstaff, 2015):

$$Wr = Wd / Wi \times 100\%.$$

References:

Ghobril, C., and Grinstaff, M.W. (2015). The chemistry and engineering of polymeric hydrogel adhesives for wound closure: a tutorial. CHEM SOC REV *44*, 1820-1835.

Li, Z., Yang, J., Li, K., Zhu, L., and Tang, W. (2017). Fabrication of PDMS microfluidic devices with 3D wax jetting. RSC ADV *7*, 3313-3320.