Supporting information for

Baicalein nanoparticle-embedded mucoadhesive hydrogel for synergistic anti-inflammation therapy in ulcerative colitis

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Determination of drug loading capacity in BZH NPs

The freeze-dried BZH NPs were dissolved in dimethyl sulfoxide (DMSO), and the absorbance at 324 nm was measured by ultraviolet–visible (UV-Vis) spectroscopy to calculate the drug-loading capacity. All experiments were performed in triplicate. The drug-loading content (DLC) was calculated using the following formula:

$$\mathrm{DLC\%} = \frac{\text{amount of BA in nanoparticles}}{\text{amount of nanoparticles}} \times 100\%$$

Rheological testing

The hydrogels were rheologically characterized using a rotational rheometer (MCR 301, Anton Paar, Austria) equipped with a parallel-plate geometry (PP-50; diameter 50 mm) at a gap of 1 mm. First, a dynamic frequency sweep was performed at a constant strain of 1% over an angular frequency (ω) range of 0.1–100 rad/s. Subsequently, to evaluate their self-healing properties, an alternating strain test was conducted by cycling the strain amplitude between 1% (low strain) and 100% (high strain).

Tissue adhesion property

A piece of porcine skin was cut and cleaned with Ethanol and PBS. Then, 1 mL of HCE stained with Rhodamine B and BZH@HCE hydrogel was injected onto the porcine skin. After 3 min, stresses were induced by invert, stretch, twist, and compress to examine the adhesion properties on the skin.

Lap shear adhesion strength test

The lap shear adhesion strength of the HCE and BZH@HCE hydrogels to fresh wet porcine skin was subjected to the lap shear adhesion test. The hydrogel with a size of 15 mm × 15 mm was adhered to one end of a porcine skin substrate. The other sheet of porcine skin was overlapped on the hydrogel side of the sheet immediately for 3 min at room temperature and then left for 180 min. The adhesion strength was measured on the universal testing machine at a cross-head speed of 5 mm/min. The shear strength was calculated using the following formula:

$$Shear\ strength = \frac{F}{A}$$

, where F is the maximum load and A is the contact area of the hydrogel with tissues.

Cytocompatibility

Rat intestinal crypt epithelial cells (IEC-6) and mouse monocyte/macrophage leukemia cells (RAW 264.7) were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, USA) at 37 °C in a humidified 5% CO_2 atmosphere (Thermo Fisher Scientific, USA). Cytocompatibility of BZH NPs and BZH NPs/HCE was evaluated in both cell lines using the CCK-8 assay and calcein-AM/propidium iodide (PI) live/dead staining.

For the CCK-8 assay, cells were seeded in 96-well plates at 1×10^5 cells per well and incubated for 24 h with BZH NPs or BZH NPs/HCE providing BA at 5, 10, 20, 40 or 100 μg/mL. CCK-8 reagent was then added and, after 2 h, absorbance at 450 nm was recorded on a microplate reader (MK3; Thermo Fisher Scientific, USA). Cell viability (%) relative to untreated controls was calculated.

For live/dead staining, cells were co-cultured with the materials for 24 h, washed with PBS, and stained with the calcein-AM/PI kit (Beyotime, China) according to the manufacturer's instructions. Live (green) and dead (red) cells were imaged immediately under a fluorescence microscope.

In Vivo Retention and Biodistribution

Fluorescein isothiocyanate (FITC)-labeled BZH/HCE@FITC were orally administered by gavage at a BA-equivalent dose of 20 mg/kg. Mice were euthanized at 2, 4, 12, and 24 hours post-administration, and their gastrointestinal tracts and major organs were harvested. Ex vivo imaging of the isolated organs was conducted using an in vivo imaging system (IVIS Spectrum, PerkinElmer, USA) to analyze the retention effect of BZH/HCE@FITC. Furthermore, colon tissues were collected for cryosectioning. After counterstaining the nuclei with DAPI, the distribution of BZH NPs within the colon tissue was examined using a fluorescence microscope (DMi8, Leica, Germany).

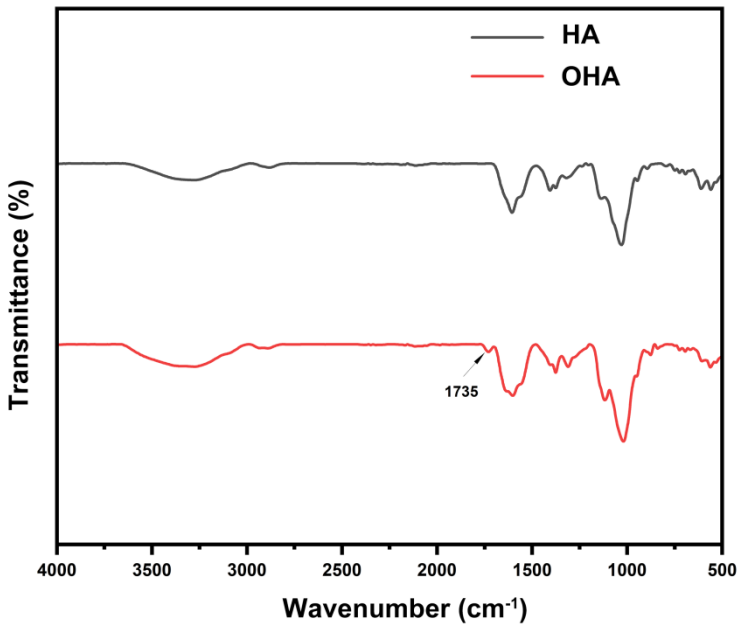

Figure S1. FT-IR spectra of HA and OHA.

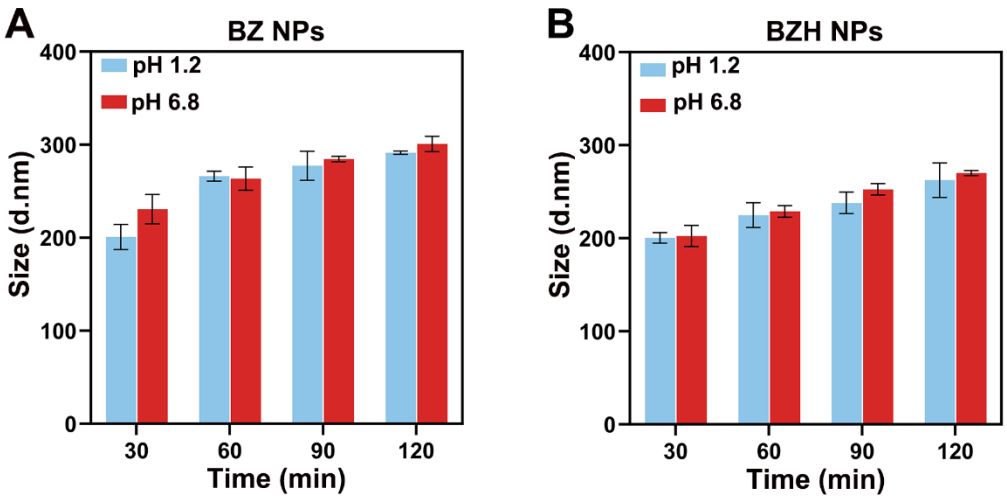

Figure S2. The effect of in vitro simulated gastrointestinal digestion on the stability of BZ (A) and BZH NPs (B).

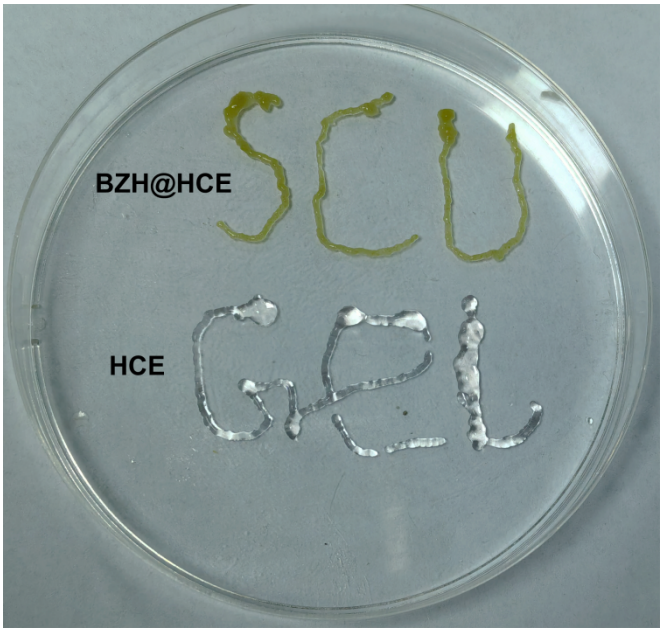

Figure S3. Injectability testing demonstrated that the hydrogel could be smoothly extruded through syringe needle (0.5 mm inner diameter) and maintain its structural integrity after extrusion.

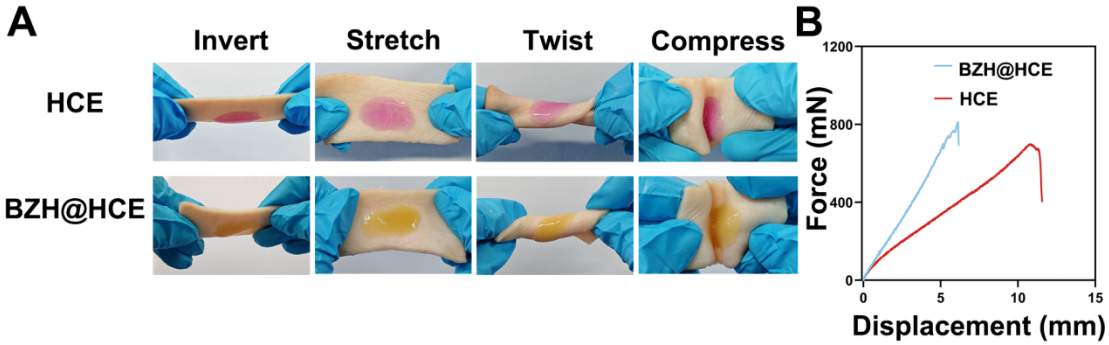

Figure S4. Adhesion performance of HCE and BZH@HCE. (A) Inverting, stretching, twisting, and compressing of HCE and BZH@HCE. (B) Adhesion-displacement curve of hydrogel on porcine skin.

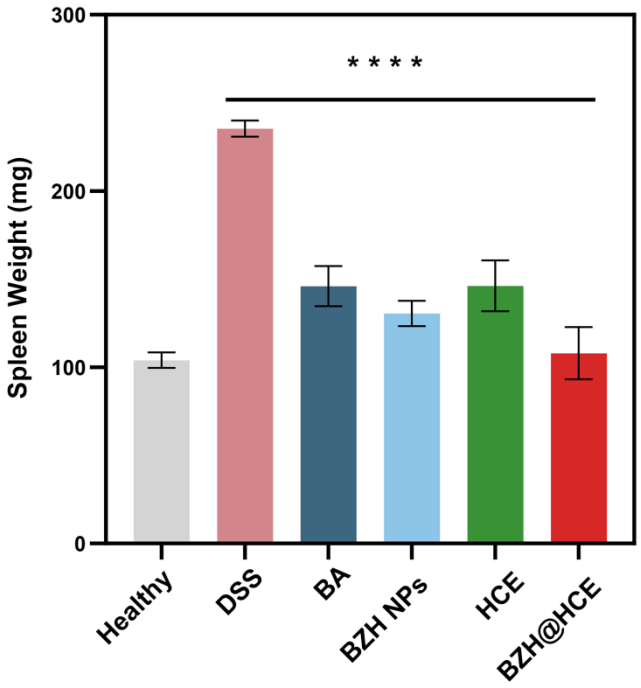

Figure S5. Spleen weights at the end of therapy.

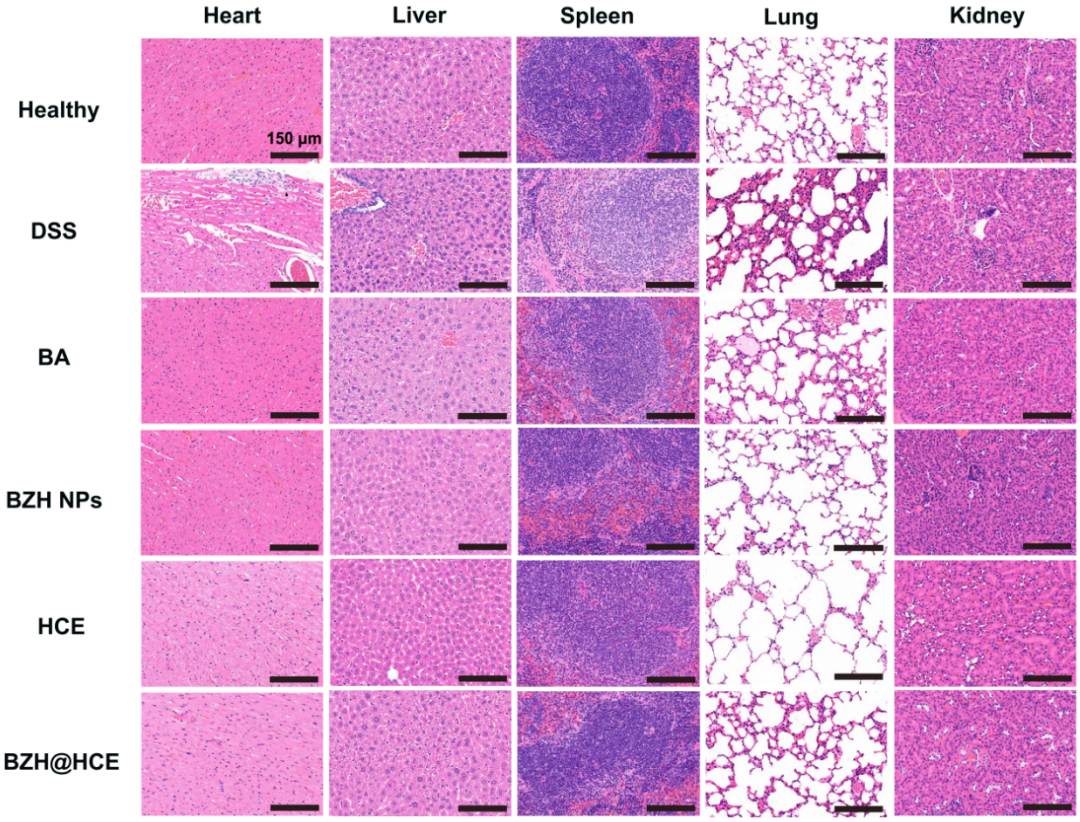

Figure S6. H&E staining of tissues from the major organs.