Supporting Information

Nanocatalytic Membrane with Sono-Responsive Antibacterial Therapy (SRAT) for Rapid Sterilization and Enhanced Chronic Wound Healing

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Materials and Methods

Preparation of PN-bioHJs

A modified liquid exfoliation technique was used to synthesize the BP nanosheets. Briefly, bulk BP crystals were dispersed in N-Methyl-2-pyrrolidone (NMP) solution (1 mg/mL) and then sonicated with a sonic tip in an ice bath for 16 h (500 W, on/off cycle: 5 s/5 s). The resulting suspension was first centrifuged at 4000 rpm lasting 15 minutes to collect the supernatant, and then the BP nanosheets were further isolated by centrifugation at 14,000 rpm for 15 minutes and subsequently stored at 4 °C for later use.

A mixture of BP nanosheets (1 mg/mL) and BTO solution (10 mg/mL) was agitated for one hour at ambient temperature before being moved to a 100 mL high-pressure reactor for three hours of hydrothermal treatment at 120 °C. The prepared BTO/BP nanohybrids were cleaned and freeze-dried at -20 °C in a vacuum lasting 12 h (named BB).

Preparation of Electrospun Nanocatalytic Membrane

1 g PLGA (Solarbio, Mn = 80,000) were mixed for 12 h in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 10 mL). The air gap distance was set to 20 cm, the electrically spun mixed liquid' injection rate was preprogrammed to 0.02 mL/min, and the electric voltage was set to 15.0 kV. Next, BTO/BP was modified onto the PLGA membranes using mussel-inspired polydopamine (PDA) chemistry (named P-BB). The PLGA fiber was immersed in a 2.0 g/L dopamine (DA) hydrochloride solution with pH = 8.0 and the membranes were transferred to a shaking table for 24 hours to facilitate the

formation of a brown polydopamine (PDA) coating on their surface. Following this, the membrane was washed with deionized (D.I.) water, and then drying at room temperature for 5 hours. The resulting membrane (10 mm × 10 mm) was then immersed in the prepared BB suspension (100 mg/mL).

Materials Characterization

The general structures of PN-bioHJs and membranes were identified through FE-SEM (JSM-7610F, JEOL, Japan). XRD (Rigaku, Japan) was applied to investigate the phase components of materials. The surface chemistry of materials was examined *via* XPS (ESCALAB 250 equipment, Thermo, USA).

Sono-Piezodynamic Performance Measurement

ROS Detection

To detect the $^1O_2$ generated by the material being examined, DPBF was utilized as the $^1O_2$ trapping agent. To make the mixed solution, 30 μM 1,3-Diphenylisobenzofuran (DPBF, Chron Chemicals, Chengdu, China) was added with 3 mL of ethanol. After placing the material in the EP tube, DPBF (200 μL) mixture was introduced. The spectra of the DPBF mixture was recorded by UV-vis spectrophotometer (UV1800PC, AoE, China) after 10 minutes of ultrasonic irradiation (1.5 W/cm^2, 50% duty cycle, 1 MHz).

To detect the ·OH generated by the material being examined, 6.6 mg terephthalic acid (TA) was added to 20 mM NaOH solution. After placing the material in the EP tube, TA (100 μL) mixture was introduced with different US time. The presence of ·OH was confirmed by the fluorescence intensity of TA at 430 nm by fluorescence spectrophotometer (F-7000, Hitachi, Japan). To detect the $\cdot O_2^-$ generated by the

material being examined, DHE was utilized as the $\cdot O_2^-$ trapping agent. 50 μg DHE was dissolved in 2 mL DMSO while 5 mg/mL DNA solution was prepared. For testing, 1 mL of 200 μg/mL sample, 150 μL DNA (5 g/L) and 10 μL DHE (25 mg/L) were added to configure the working solution. The product 2-hydroxy ethidium was tested for fluorescence signal at 625 nm using sonication (1.5 W/cm^2) with different ultrasound time through fluorescence spectrophotometer (F-7000, Hitachi, Japan). The ultrasound parameters for all ROS detection experiments were set to 1MHz, 50% duty cycle, and 1.5 W/cm^2.

The ESR (JES-FA200, JEOL, Japan) was tested for identifying the manufacturing of ROS. Following ultrasonic treatment (1.5 W/cm^2, 50% duty cycle, 1 MHz) of the membranes for different time intervals (0, 5, and 10 min), the O_2^- and ·OH were identified using DMPO (Chron Chemicals) reagent, and $^1O_2$ was obtained using TEMP (Chron Chemicals) reagent.

In vitro **Assessment of Antimicrobial Activity**

Spread Plate Technique

To evaluate the antibacterial properties of P-BB under different ultrasound conditions, membrane squares (1×1 cm^2) were placed into individual wells of a 48-well plate, and 500 μL of a bacterial solution (1×10^5 CFU/mL) of *E. coli* (ATCC 25922) was added to each well. The wells were then subjected to different ultrasound treatments based on the experimental groups: Group 1 (control) received no ultrasound exposure, while other groups were exposed to varying frequencies (1 MHz, 2 MHz, and 3 MHz), intensities (0.5 W/cm^2, 1.5 W/cm^2, and 2.5 W/cm^2), and exposure times (5, 10, and 15

minutes). After treatment, 10 μL of the bacterial solution from each well was plated onto LB agar plates and incubated at 37 °C for 24 hours to allow bacterial colony growth. The number of colonies was then counted to determine the antibacterial efficacy of P-BB under each ultrasound condition. The inhibition rate was calculated by comparing the bacterial growth in each experimental group to the control group, allowing for the assessment of the impact of different ultrasound parameters on antibacterial activity.

The antimicrobial properties of various materials (PLGA, P-BTO, P-BP, and P-BB) against microbial strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) was investigated by the spread plate technique. Membrane squares measuring 1×1 cm^2 were introduced into 48-well plates, and PLGA group was applied as the control group. A bacteriological solution of 500 μL, comprising 1×10^5 CFU/mL and diluted in lysogeny broth (LB) medium, was introduced into each well alongside the samples. This was succeeded by subjecting the setup to either 10 min of ultrasonic irradiation (1.5 W/cm^2, 50% duty cycle, 1 MHz) or maintaining a state of silence. Following that, 10 μL of microbial culture was taken from every well and put to LB agar plates for 24 h at 37 °C to achieve colonies of bacterium. The sterilizing rate (%) of the samples was identified *via* the next equation:

$$\text{Antibacterial Activity (\%)} = [(N_{blank} - N_{sample}) / N_{blank}] \times 100\ \%$$

Here, N_{blank} signifies the residual count of bacterial cells within the PLGA samples untouched by ultrasonic radiation, whereas N_{sample} denotes the count of bacterial cells after diverse treatments.

Examination of Bacteria Morphology

The structures of bacteria after treating with various membranes were identified *via* FE-SEM. Membrane was mixed onto the cell slides, and these slides were incubated with bacteria suspension under US (1.5 W/cm^2, 50% duty cycle, 1 MHz) or in the silence for 10 min. The remaining bacterial suspension was taken out, and the samples were fixed with glutaraldehyde (Sino Biological, Beijing, China). Then, the bacteria were gradually dehydrated using a graded alcohol series (30–100%) and then dried through critical point drying for morphology observation *via* FE-SEM.

LIVE/DEAD Staining

Bacteriological solution (1 mL, 1×10^8 CFU/mL) were cultured with various materials lasting 12 h at 37 °C with continual shaking before receiving treatment with US (1.5 W/cm^2, 50% duty cycle, 1 MHz) or in silence lasting 10 min. Next, the materials were rinsed several times by PBS. Each well received a colored combination of SYTO 9 and propidium iodide (PI, 200 μL) and was stained lasting 15 min using the LIVE/DEAD staining kit (Beijing Solarbio Science & Technology Co.,Ltd, Beijing). Microbes in every group were photographed using CLSM (N-SIM, Nikon, Japan) after staining.

Investigation for Anti-Biofilm Capacity

Formation of Biofilms

S. aureus (1×10^8 CFU/mL, 500 μL) was cultured with glass coverslips in a 48-well plate. Incubation was carried out at 37 °C under continuous shaking to promote biofilm formation. At 24-hour intervals, the media were removed, and the coverslips were

rinsed by sterile PBS and then taken the place of with fresh LB media. After the passage of 4 days, fully formed biofilms were successfully cultivated and collected.

Biofilm Elimination Analysis

For the investigation of *in vitro* antibiofilm efficacy of materials, well-established mature biofilms of *S. aureus* were cultured with various membrane co-cultures. Subsequent to this, the biofilm samples underwent either ultrasonic treatment (1.5 W/cm^2, 50% duty cycle, 1 MHz, 10 min) or were incubated in an undisturbed state of silence. The biofilms were then washed with PBS. For further study, the residual biofilms were dyed with crystal violet. A scanner was used to collect the photos of the biofilms for qualitative examination. To dissolve the dye deposited on the biofilms for quantification, an ethanol solution was used. As for quantitative measurement, eluting the dye adsorbed on the biofilms with an ethanol solution was used and measuring the OD at 570 nm.

Moreover, LIVE/DEAD Kit was used to stain the residual biofilms for evaluating the viability of the remaining microbes. Before incubating in darkness for at least 15 min, a 200 μL colored combination of SYTO 9 for nucleic acid staining and PI was applied to every well. Following that, the stained biofilms was detected by CLSM.

Biocompatibility Investigation *In Vitro*

Cytotoxicity examination

L929 cells (American Type Culture Collection) was incubated by the Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum at 37 °C in a humidified atmosphere. Cytotoxicity of materials was determined by CCK-8 (Dojindo,

Japan). All membranes were sterilized under UV light for around 30 min before cell culture. L929 cells (1×10^4/well) were grown on a 48-well plate lasting 6 h. Following that, several samples (PLGA, P-BTO, P-BP, and P-BB, 1×1 cm^2) were added to every well and cells were grown. On days 1, 3, and 5, the medium was replaced with 330 μL of a combination CCK-8 solution. After an hour of incubation, the samples' absorbance at 450 nm was measured. Subsequently, the culture was replenished with fresh medium to facilitate continued cell cultivation.

L929 cells (1×10^4 cells/well) were cultured in a 48-well plate with DMEM supplemented with 10% fetal bovine serum, and incubated at 37 °C in a humidified atmosphere. Prior to cell seeding, all P-BB membrane samples (1×1 cm^2) were sterilized under UV light for 30 minutes. After 6 hours of cell attachment, the P-BB samples were added to each well, corresponding to the ten experimental groups with different ultrasound parameters: Group 1 (control) received no ultrasound treatment, while the other groups were exposed to varying frequencies (1 MHz, 2 MHz, and 3 MHz), intensities (0.5 W/cm^2, 1.5 W/cm^2, and 2.5 W/cm^2), and exposure times (5, 10, and 15 minutes). On days 1, 3, and 5, 330 μL of CCK-8 solution was added to each well to assess cytotoxicity. After an hour of incubation, the absorbance at 450 nm was measured to determine cell viability. The medium was then replaced with fresh medium to allow continued cell culture for the following days.

Observation of Cell Structure and Cytoskeleton

L929 cells (2×10^4 cells / well) were grown on a 48-well plate lasting 6 h at 37 °C. Following that, several samples (PLGA, P-BTO, P-BP, and P-BB, 1×1 cm^2) were added

to every well and cells were grown for morphological analysis. The cells were fixed for 2 hours in 300 μL of 2.5% glutaraldehyde solution and subsequently dehydrated through a graded ethanol series (30% to 100%) with 20 minutes for each step. After drying, FE-SEM was used to investigate the samples.

Immunofluorescence labeling was applied to determine the cytoskeleton status of L929 cells treated by membrane. A volume of 500 μL of L929 cells (1×10^4 / well) was cultured in a 48-well plate including glass coverslips. Next, cells were also fixed for 0.5 h in 500 μL of 4% paraformaldehyde before being rinsed by PBS. Triton X-100 (0.1%) was used to permeabilize the pro-fixed cells lasting 20 min before being rinsed by PBS. The cellular cytoskeleton was subjected to staining using FITC-phalloidin (Solarbio), while the nuclei were stained utilizing DAPI (Solarbio). CLSM examined all of the samples to acquire fluorescence pictures. Similarly, Live & dead staining was performed, and photographs were obtained using an inverted fluorescence micro scope.

Antimicrobial and Wound Rehabilitation *In Vivo*

Infection Model of Mouse Skin Trauma

To investigate the fibers' antibacterial properties, we established the infected wound models. Female Kunming mice (6–8 weeks) bought from Beijing HFK Bioscience Co., Ltd were selected for animal experiments, and all the animal progress followed the animal ethical standard from Animal Ethics Committee in West China Hospital, Sichuan University, Chengdu, China (20240704003). The infected wound model was established through the following steps: 1) excision of a circular area approximately 8 mm in diameter from the epidermal layer; 2) introduction of 20 uL of *S. aureus* on the

wounded area and incubation for 1 day; 3) mice assigned to different groups received indicated fabrics treatment through wrapping the fabric around the surface of the wound and ultrasonic treatment (1.5 W/cm^2, 50% duty cycle, 1 MHz) by applying sound waves to the surface of the wound using an ultrasound machine for 15 min; 4) the fabrics were removed 72 h later and the wounded area recorded every other day. The infection models were divided into five groups according to receiving ultrasonic treatment or not: PLGA-, PLGA+, P-BB- and P-BB+ (n = 5). 3M wounds dressing was utilized for sealing the area that was infected and restricting its exposure to oxygen for 1 day in order to generate an anoxic condition. Following that, tissue fluid from every mouse's incision was taken and stored in PBS (1 mL) for antibacterial counting test. The mice were euthanized on the ninth day after surgery, and the relevant wound tissues, as well as important organs (heart, spleen, lung, liver, and kidney), were carefully collected for histological evaluation.

In Vivo Healing Assessment

Photographs were captured daily to record the changing wound areas for assessing the healing progression.

In Vivo Antibacterial Test

The wound-adjacent tissue fluid was incubated in PBS for one day. Following this, LB agar plates were used to incubate bacterial solution (100 μL) and subsequently cultured at 37 °C lasting 24 h to ascertain bacterial count.

$$\text{Antibacterial Activity (\%)} = [(N_{blank} - N_{sample}) / N_{blank}] \times 100\ \%$$

Here, N_{blank} signifies the residual count of bacterial cells within the PLGA- group, whereas N_{sample} denotes the count of bacterial cells in other groups.

Blood Routine Examination

Upon the conclusion of the mouse experiments, blood routine tests were performed to assess the physiological impact of the treatments. Representative individuals were randomly selected from each experimental group to ensure robust and representative results. Strict aseptic techniques were employed for the preparation of blood collection tools, including syringes and needles. The collected blood was transferred into sterile blood collection tubes, and the samples were gently mixed to ensure uniformity. The blood were subsequently used for routine analysis, which included the following indicators: neutrophil count (NEUT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hematocrit (HCT), monocyte percentage (MONO%), hemoglobin level (HGB), red blood cell count (RBC), platelet count (PLT), mean corpuscular volume (MCV), platelet distribution width (PDW), lymphocyte percentage (LYMPH%), red cell distribution width - coefficient of variation (RDW-CV), neutrophil percentage (NEUT%), monocyte count (MONO#), lymphocyte count (LYMPH#), platelet crit (PCT), white blood cell count (WBC), and mean platelet volume (MPV).

Histopathologic Examination

The gathered skin tissues were carefully collected and subsequently pro-fixed using 4% paraformaldehyde. After fixation, the tissues were paraffin-embedded and then sectioned into slices. H&E staining was performed to facilitate observation of

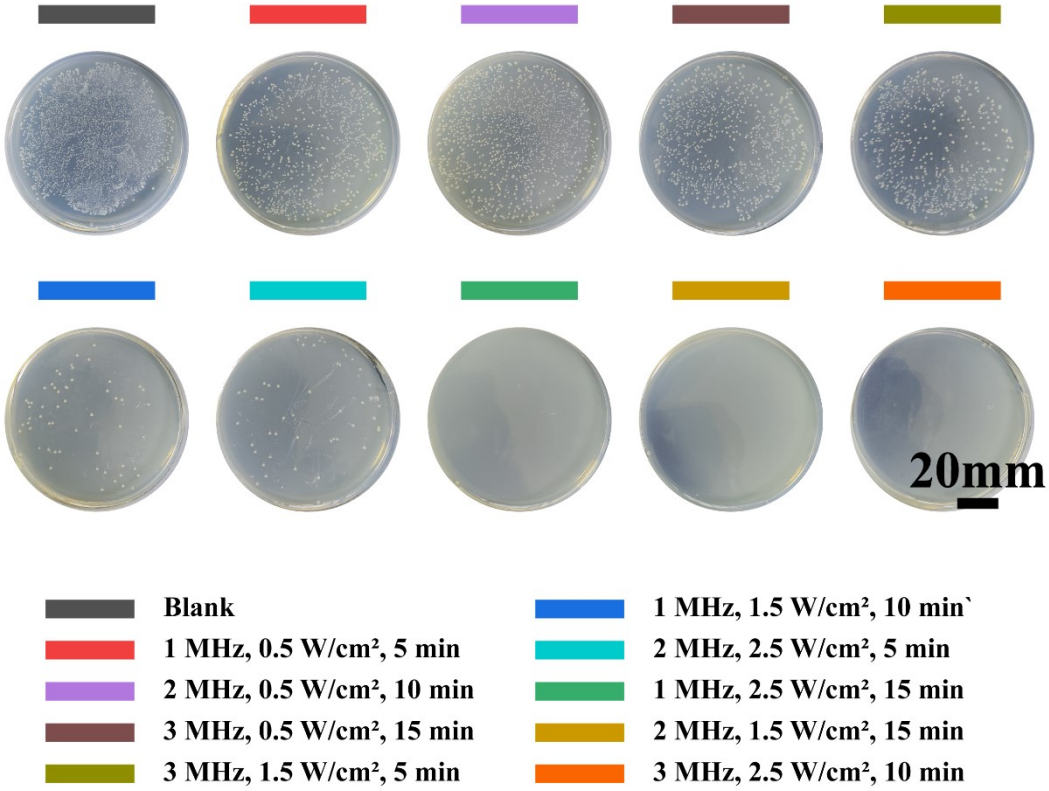
contaminated tissues. In addition, key organs were subjected to H&E staining to assess in vivo biocompatibility. The deposition of collagen fibers in the wound was tested by Masson staining. Immunohistochemical staining was performed for TNF-α and CD31. Immunofluorescence labeling was also used to assess VEGF and IL-6. Image J software was employed for calculating the findings of the histochemical study.

Captions of Figures

Figure S1. Images of microbe populations of *E. coli* treated with P-BB on different ultrasound condition.

Figure S2. CCK-8 results of L929 fibroblasts treated with P-BB on different ultrasound condition over 1, 3, and 5 days.

Figure S3. H&E staining images of heart, liver, spleen, lung, and kidney of mice from the different groups after 9 days treatment.



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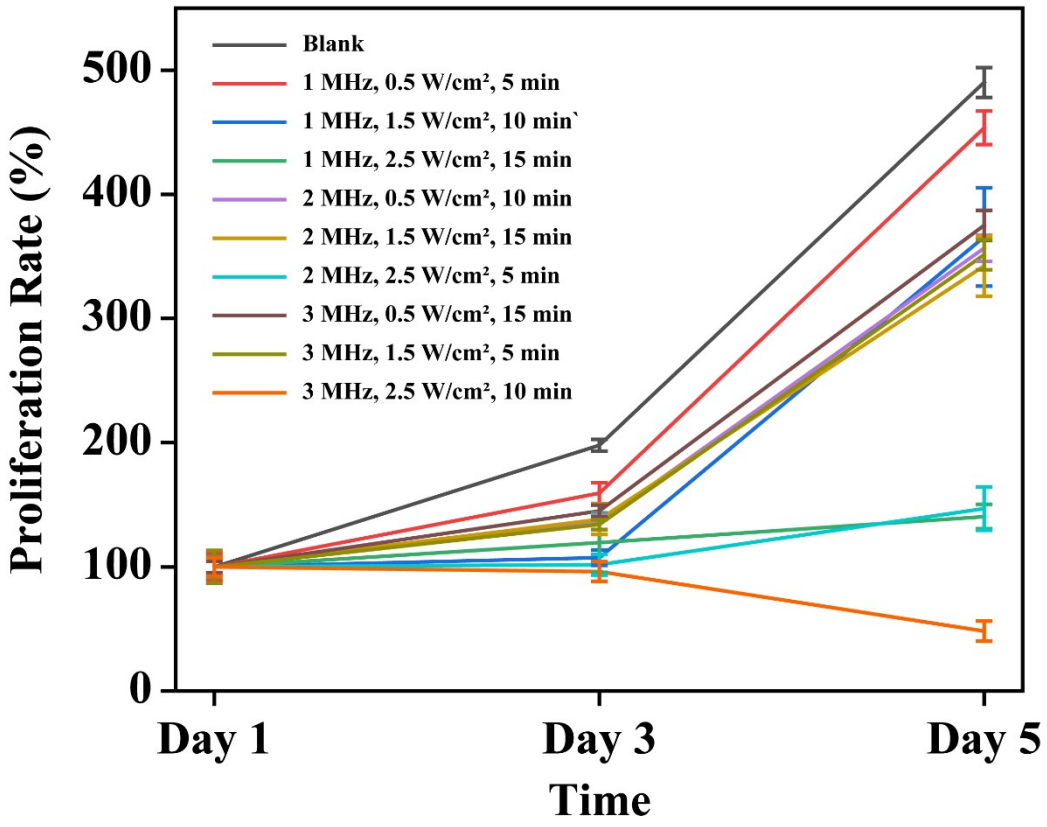

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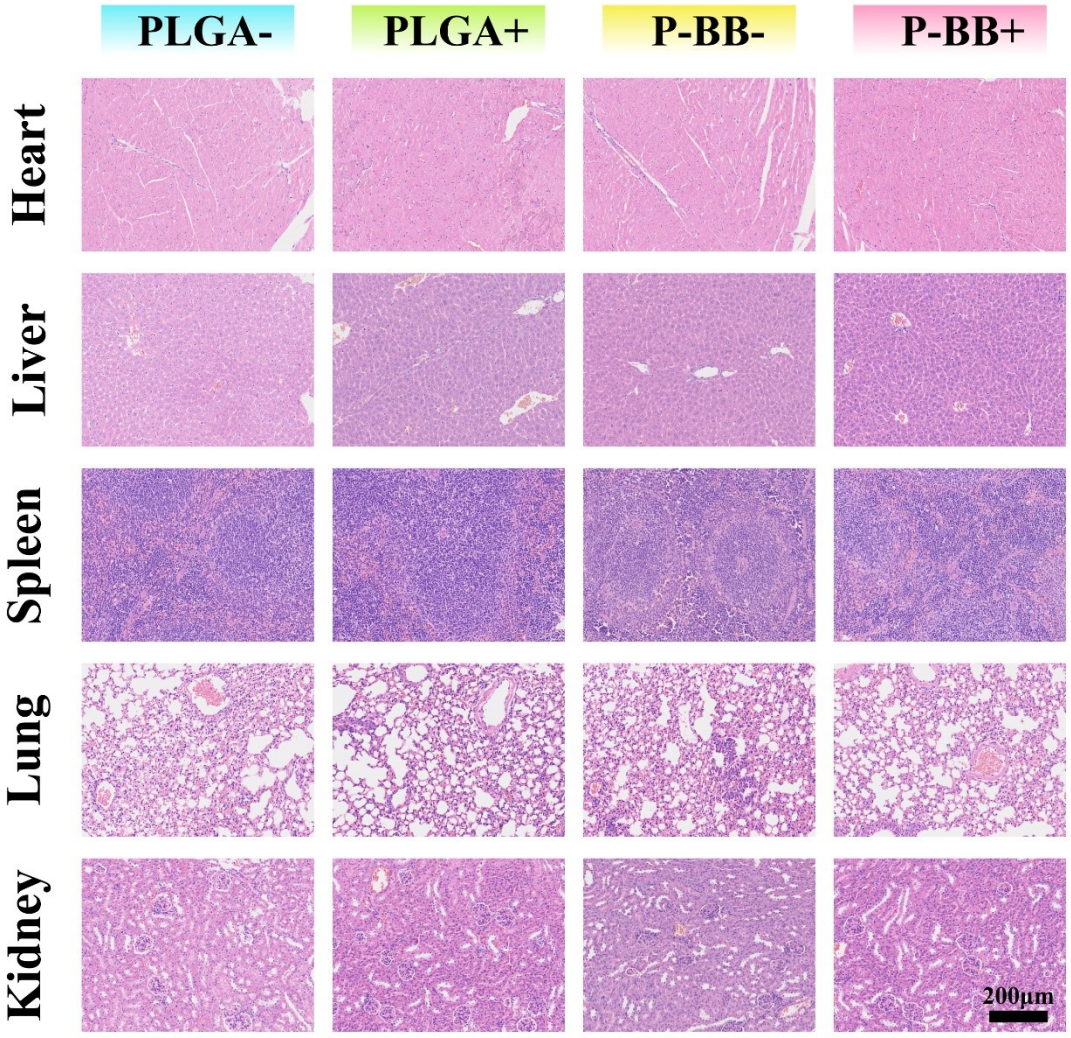

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