Supporting Information for

Localized and Sustained Delivery of Indomethacin using Poly (lactic-co-glycolic acid)-Based Microspheres to Prevent Traumatic Heterotopic Ossification

Supporting Information:

1. Materials and methods

In Vitro Degradation Evaluation of INDO-PLGA MPs

To evaluate the degradation behavior of INDO-PLGA MPs, an in vitro degradation assay was conducted under physiological and inflammatory-mimicking conditions. 30% INDO-PLGA MPs were prepared and lyophilized prior to the degradation study. A known quantity of microspheres (~10 mg) was accurately weighed and suspended in 5 mL of phosphate-buffered saline (PBS, pH 7.4) to simulate physiological conditions. To model an inflammatory environment, hydrogen peroxide (H_2O_2, 1 mM) was introduced into a parallel set of samples, mimicking the oxidative stress present in inflamed tissues. Each sample was incubated at 37°C with gentle agitation (50 rpm) in a shaking incubator to maintain homogeneous conditions. At predetermined time points (Days 0, 3, 7, 14, 21, and 28), microsphere samples were collected, centrifuged at 12,000 rpm for 10 min, and washed twice with deionized water to remove residual salts. The samples were then lyophilized and weighed to determine mass loss over time.

Rat Calvarial Defect Model

Male Sprague-Dawley (SD) rats (8–10 weeks old, weighing 250–300 g) were used for the calvarial defect model. All rats were anesthetized using intraperitoneal injection of pentobarbital sodium (40 mg/kg body weight). The surgical area was shaved and disinfected with povidone-iodine, followed by a midline sagittal incision on the dorsal calvarium to expose the skull. Using a low-speed dental drill (with a 4-mm trephine bur) under constant saline irrigation, a standardized unilateral full-thickness calvarial defect (4 mm in diameter) was created in the parietal bone, ensuring the periosteum was carefully preserved to minimize additional trauma. Care was taken to avoid damaging the dura mater during defect creation. The INDO-PLGA MPs were implanted in the defect side. After implantation, the soft tissue was carefully repositioned and sutured. At 4 weeks post-surgery, micro-CT analysis was performed to evaluate the bone healing.

2. Results Figure

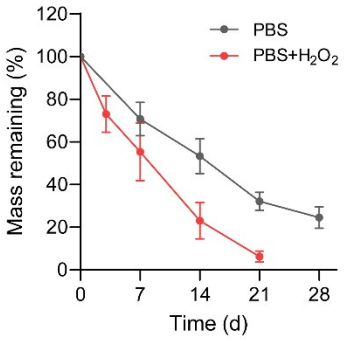

Figure S1. In vitro degradation evaluation of INDO-PLGA MPs

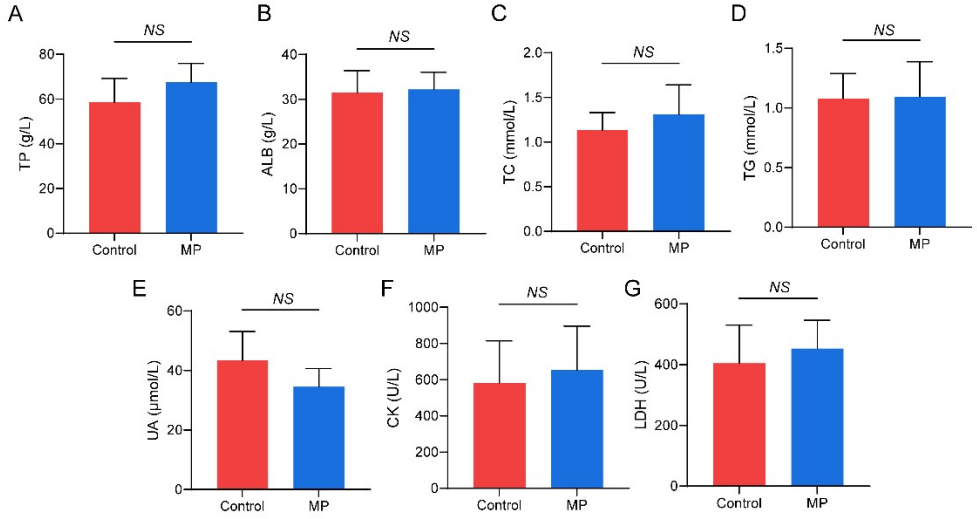

Figure S2. Quantitative blood biochemical analysis, including A) TP, B) ALB, C) TC, D) TG, E) UA, F) CK, and G) LDH. (n=3 per group).

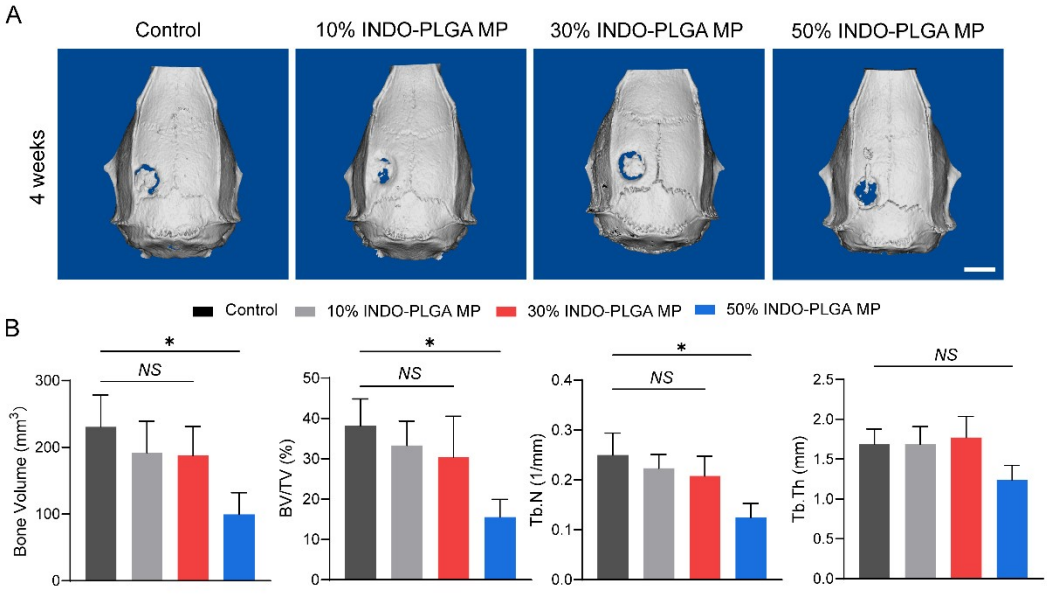

Figure S3. Micro-CT evaluation of bone healing in calvarial defect model. A) 3D-reconstructed micro-CT images of the calvarial defect at 4 weeks post-surgery. Scale bar: 5mm. B) Quantitative analysis of newly formed bone in the calvarial defect area. (n=3 per group).

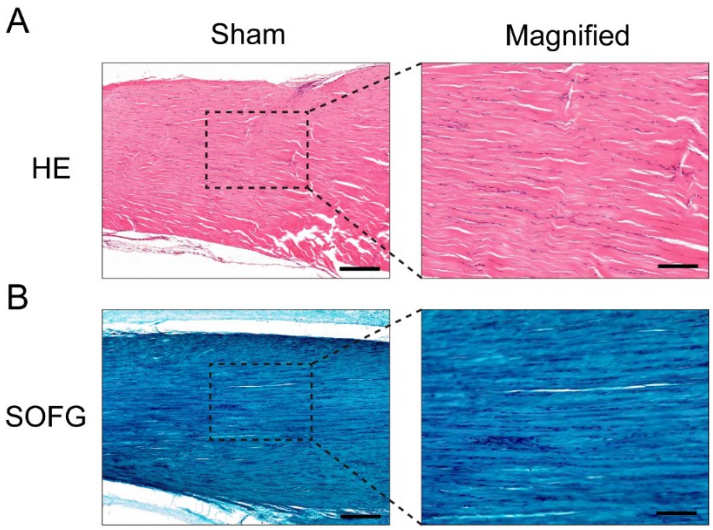

Figure S4. A). HE staining and corresponding local magnified views of normal Achilles tendon sections. Scale bar: 500μm and 200μm. B) SOFG staining and corresponding local magnified views of normal Achilles tendon sections. Scale bar: 500μm and 200μm.