Supporting Information

Bioinspired design of hierarchical structured hydrogels with extraordinary lubrication and load-bearing capacity

Xiaoduo Zhao[a†], Xinyuan Kou[a†], Weiyi Zhao[a], Yibo Chen[a], Yunsong Kong[a], Jinshuai Zhang[b,c*], Zhengfeng Ma[a*], Yunlei Zhang[a], Bo Yu[a], Shuanhong Ma[a*]

a. State Key Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, 730000, China

b. Shandong Laboratory of Advanced Materials and Green Manufacturing at Yantai, Yantai, 264006, China

c. Yantai ZhongkeResearch Institute of Advanced Materials and Green Chemical Engineering, Yantai, 264006, China

† Xiaoduo Zhao and Xinyuan Kou contributed equally to this work.

Experimental section

Materials and methods

a) Materials

Poly(vinyl alcohol) (PVA) with a weight-average molecular weight (M_x) of 89–98 kDa and a degree of hydrolysis of 99% was purchased from Sigma–Aldrich. Dimethyl sulfoxide (DMSO, anhydrous, ≥99.9%) was obtained from Sigma–Aldrich. Fetal bovine serum (FBS; Cellmax, Cat. No. SA311) was used for cell culture supplementation. Dulbecco's Modified Eagle Medium (DMEM; Gibco, Cat. No. C11960500BT) served as the basal culture medium. Phosphate-buffered saline (PBS; Solarbio, Cat. No. P1020) was used for cell washing. Cell proliferation and cytotoxicity were assessed using the Cell Counting Kit-8 (CCK-8; Solarbio, Cat. No. CA1210). Deionized water was purified in the laboratory and used throughout the experiments. All reagents were of analytical grade and used as received unless otherwise specified. Fresh chicken joints were purchased from a local butcher shop.

b) Preparation of the Hydrogels

PVA hydrogels with spontaneous tensile orientation under confinement (STOC) were fabricated via a three-step process. First, PVA powder was dissolved in DMSO at 90 °C under vigorous mechanical stirring to prepare a 20 wt% homogeneous solution. After degassing by high-speed centrifugation, the solution was cast into a polytetrafluoroethylene (PTFE) mold and allowed to stand at room temperature for 24 h to form a mechanically weak physical hydrogel through preliminary chain entanglement and hydrogen bonding.

In the second step, the as-prepared hydrogel was uniaxially stretched to predetermined ratios using a custom-designed fixture. The pre-stretching ratio (λ) is defined as $\lambda = (L - L_0)/L_0 \times 100\%$, where L_0 and L are the original and stretched lengths, respectively. The prepared samples were denoted as S-x-PVA, where x represented the pre-stretch ratio, x=50%-300%. While maintained under fixed strain, the stretched hydrogel was immersed in deionized water at room temperature for 48 h to simultaneously induce solvent exchange and stabilize chain orientation, with the water refreshed every 6 h to ensure complete removal of DMSO.

In the final step, the hydrogel (still fixed in the stretching fixture) was thermally annealed at 120 °C for 1 h, followed by removal from the fixture and equilibration in deionized water to achieve swelling equilibrium. The resulting hydrogels were denoted as STOC.

To introduce superior surface lubrication, DMSO preheated to 90 °C was applied to the surface of the STOC hydrogel for varying times (15 s, 30 s,45 s), after which the sample was immediately immersed in deionized water to remove residual DMSO, yielding surface-dissociated hydrogels designated as D-STOC. For comparison, two control samples were prepared using similar procedures. The hydrogel obtained after only the first solvent-exchange step (without stretching or thermal annealing) was designated as pristine PVA. Hydrogels that underwent stretching followed by thermal annealing at different temperatures were labeled A-y-PVA, where y represents the annealing temperature (100–140 °C).

c) Morphology Characterization

The surface morphology of fully swollen hydrogel samples was observed using an optical microscope (Olympus BX51, Tokyo, Japan). To visualize the internal microstructure, the swollen hydrogels were first quenched in liquid nitrogen and then freeze-dried under vacuum at −40 °C for 48 h using a freeze dryer (Scientz-20F/A, Ningbo Scientz Biotechnology Co., Ltd., China). The dried specimens were sputter-coated with a thin layer of gold and subsequently imaged with a

scanning electron microscope (SEM, Tescan Mira3, Czech).

d) Mechanical Testing

Tensile and tear mechanical properties were measured using a universal testing machine (EZ-Test, Shimadzu, Kyoto, Japan) at room temperature. For uniaxial tensile tests, hydrogel samples were cut into dumbbell-shaped specimens (35 mm × 2 mm, length × width). Tests were performed at a constant crosshead speed of 100 mm/min.

Trouser tear tests were conducted on rectangular specimens with dimensions of 45 mm (total length) × 20 mm (total width), featuring a 10 mm wide trouser leg and a 20 mm precut notch. The crosshead speed was maintained at 100 mm/min.

Surface mechanical properties were characterized by nanoindentation using a Bioindenter UNHT³ Bio system (Anton Paar, Austria) equipped with a ruby spherical indenter (radius R=0.5 mm). A maximum load of 200 μN was applied, and the surface modulus was determined from the loading–unloading curves.

e) Crystal Structure Characterization

To investigate the evolution of crystalline structures during hydrogel preparation, small-angle X-ray scattering (SAXS) and differential scanning calorimetry (DSC) measurements were performed. SAXS experiments were conducted on a Xenocs Xeuss 3.0 system (Xenocs SA, France) equipped with a Cu Kα X-ray source operating at 30 W. Scattering patterns were collected using an Eiger2 R 1M detector. The scattering vector (q) range was set from 0.002 to 0.820 nm^{-1}. One-dimensional scattering curves were obtained by sector-averaging the two-dimensional SAXS patterns. The lattice spacing (L) of the crystalline domains in the lubrication layer was calculated from the corrected scattering intensity (Iq^2) versus scattering vector (q) plots.

To prevent spontaneous secondary crystallization of amorphous polymer chains during drying, which would distort the true crystalline information of the as-prepared hydrogels, all samples underwent chemical crosslinking fixation prior to DSC analysis (STA 449 F3, NETZSCH, Germany). Briefly, fully swollen hydrogel specimens were immersed in a crosslinking solution consisting of 10 mL glutaraldehyde (50 vol%), 0.5 mL concentrated hydrochloric acid (38 wt%), and 89.5 mL deionized water for 30 min. The chemically fixed samples were then thoroughly rinsed with excess deionized water to remove residual reagents and dried in a convection oven at 37 °C for 24 h. Dried samples (≈5–10 mg) were accurately weighed, sealed in aluminum crucibles, and analyzed using a differential scanning calorimeter. Measurements were performed under a nitrogen atmosphere (flow rate 30 mL/min) from 50 °C to 270 °C at a heating rate of 10 °C/min.

f) Water Content and Surface Wettability

The water content of the hydrogels was determined by the gravimetric method. Fully swollen hydrogel samples were gently blotted with filter paper to remove surface water and weighed immediately (W_1). The samples were then freeze-dried to constant weight using a freeze dryer and reweighed (W_2). The water content (C_w) was calculated according to the following equation:

$$C_w = \frac{W_1 - W_2}{W_1} \times 100\%$$

The surface wettability of the hydrogels was evaluated by static water contact angle measurements using an optical contact angle goniometer (DSA-100, Krüss GmbH, Hamburg, Germany). A 5 μL droplet of deionized water was placed on the sample surface at room temperature, and the contact angle was recorded within 10 s. At least five independent measurements were performed at different locations on each sample, and the average value was reported.

g) Evaluation of lubrication performance

Tribological properties were evaluated using a reciprocating tribometer (TRB, CSM, Anton Paar, Switzerland). A stainless-steel pin with a diameter of 5 mm was employed as the counterpart. Deionized water was initially used as the lubricant. The reciprocating sliding tests were performed with a stroke length of 5 mm. To investigate the influence of normal load, tests were conducted under stepwise increasing loads of 0.5 N, 1 N, 2 N, 5 N, and 10 N. Similarly, the effect of sliding frequency was studied at 0.2 Hz, 0.5 Hz, 1 Hz, 2 Hz, and 5 Hz. Each test was run for 600 cycles. Four different lubricants were used in this study: phosphate-buffered saline (PBS), normal saline (NS), hyaluronic acid solution (HA), and BSA (Bovine serum albumin). Specifically, the following concentrations were used: 1 wt% HA solution, 0.1 mg/mL BSA solution, and 0.9 wt% NaCl solution. All solutions were freshly prepared with deionized water prior to testing.

To further assess the long-term lubrication performance and durability of the materials, prolonged sliding tests were carried out under a constant load of 5 N and a frequency of 1 Hz for 30,000 cycles. After the tribological tests, the wear tracks on the samples were examined using an optical microscope and SEM to analyze the surface morphology and wear characteristics.

h) Biocompatibility Evaluation

The cytotoxicity of the hydrogels was assessed using the Cell Counting Kit-8 (CCK-8) assay with L929 mouse fibroblast cells. Hydrogel extracts were prepared at concentrations of 0%, 25%, 50%, 75%, and 100% (v/v) in culture medium. L929 cells in the logarithmic growth phase were seeded into 96-well plates at a density of 5×10^3 cells per well. After cell attachment, the culture medium was replaced with hydrogel extracts, and the cells were incubated for 1, 3, 5, and 7 days. At predetermined time points, the medium was removed, and the wells were gently washed twice with phosphate-buffered saline (PBS). Then, 100 μL of fresh medium containing 10% CCK-8 reagent was added to each well. After incubation at 37 °C in a 5% CO_2 atmosphere for 4 h, the absorbance at 450 nm was measured using a microplate reader. Background absorbance was determined from wells containing only medium without cells. Cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{OD_{experimental} - OD_{background}}{OD_{control} - OD_{background}} \times 100\%$$

L929 cells in the logarithmic growth phase were seeded into confocal laser scanning microscopy dishes at a density of 1×10^5 cells/mL. After incubation with hydrogel extracts for the designated periods, the cells were stained with a live/dead assay kit (Calcein-AM/PI). Staining was performed at 37 °C for 30 min, followed by two gentle washes with PBS. Fluorescence images were acquired using a confocal laser scanning microscope. Calcein-AM (live cells, green fluorescence) was visualized at excitation/emission wavelengths of 488/517 nm, and propidium iodide (PI, dead cells, red fluorescence) was visualized at 561/580 nm. All images were captured at 200× magnification.

Animal experiments were performed in accordance with institutional animal care guidelines. All experimental animals were purchased from Wuhan Hualian Biotechnology Co., Ltd. (Experimental Animal Use License No.: SYXK (e) 2023-0104), and the animals experiments obtained the approval notice from the institutional review board (approval number: HLK-20250519-002). Hydrogel samples were prepared as discs (3 mm diameter × 1 mm thickness), photographed, and weighed before implantation. Under anesthesia, the discs were subcutaneously implanted into

the dorsal region of Sprague-Dawley (SD) rats (4-week-old, male) without damaging the underlying fascia or adipose tissue. After 7 days, the rats were euthanized. The implantation sites were visually inspected for signs of inflammation or abnormal tissue responses. The skin was incised, and photographs were taken to evaluate local tissue reactions. The implants were carefully explanted, photographed, and re-weighed. In addition, tissue sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E), then sealed with neutral gum and examined under an optical microscope.

Blood samples were collected on days 1, 4, and 7 post-implantation for ELISA quantification of pro-inflammatory cytokines TNF-α and IL-1β. On day 7, the subcutaneous tissues surrounding the implants were excised and processed for histological analysis (hematoxylin and eosin staining) to evaluate inflammatory cell infiltration and tissue compatibility.

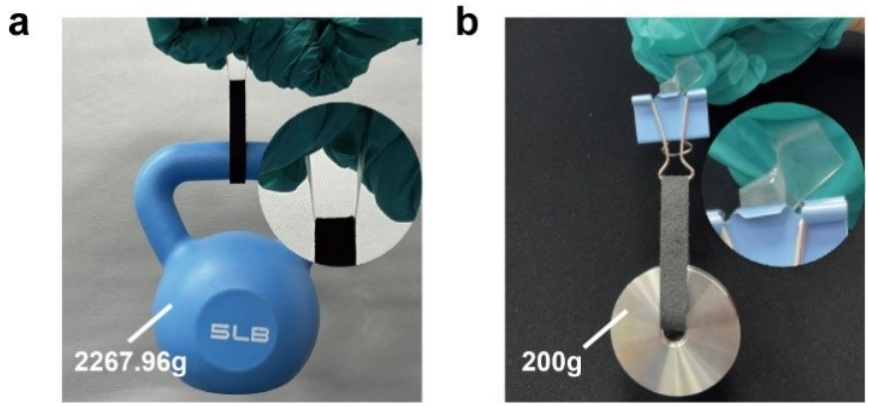

Fig. S1 Demonstration of the outstanding mechanical performance of the STOC hydrogel. (a) Load-bearing capacity and (b) tear resistance.

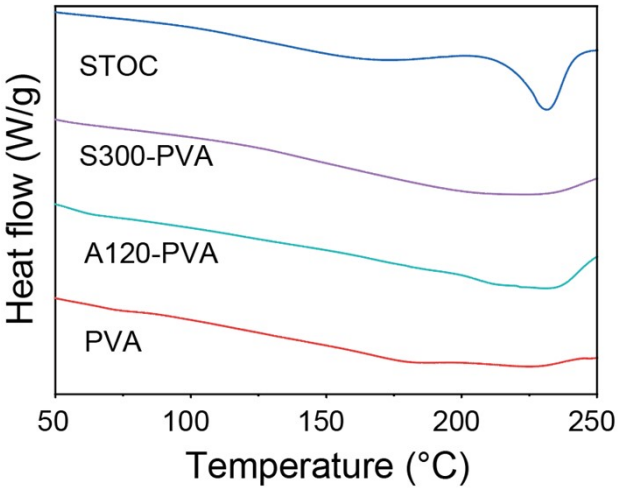

Fig. S2 DSC curves of the four representative samples.

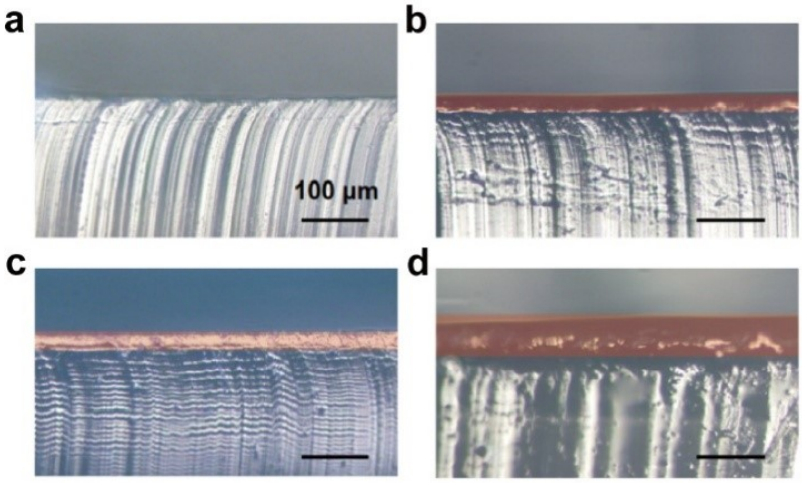

Fig. S3 Cross-sectional optical microscopy images of sample STOC-D after surface dissociation for different durations: (a) 0 s, (b) 15 s, (c) 30 s, (d) 45 s.

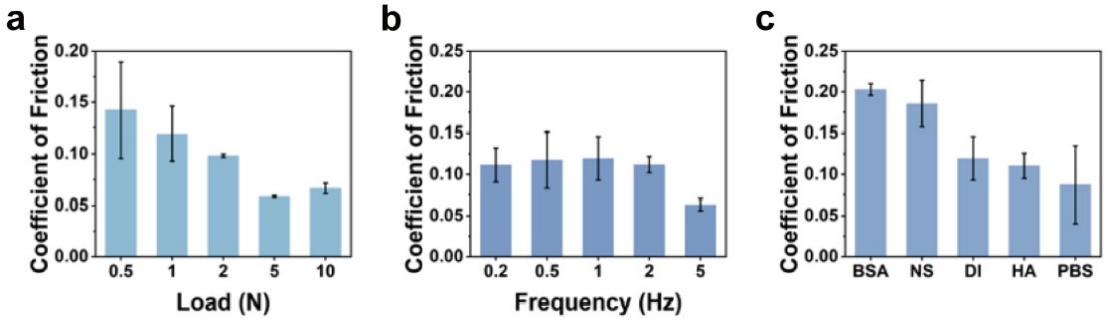

Fig. S4 Tribological performance of STOC hydrogels under various conditions. Average COF under (a)different normal loads, (b) sliding frequencies, and (c) lubricating medium. (DI: deionized water, BSA: bovine serum albumin solution, NS: normal saline, HA: hyaluronic acid solution, PBS: phosphate-buffered saline).

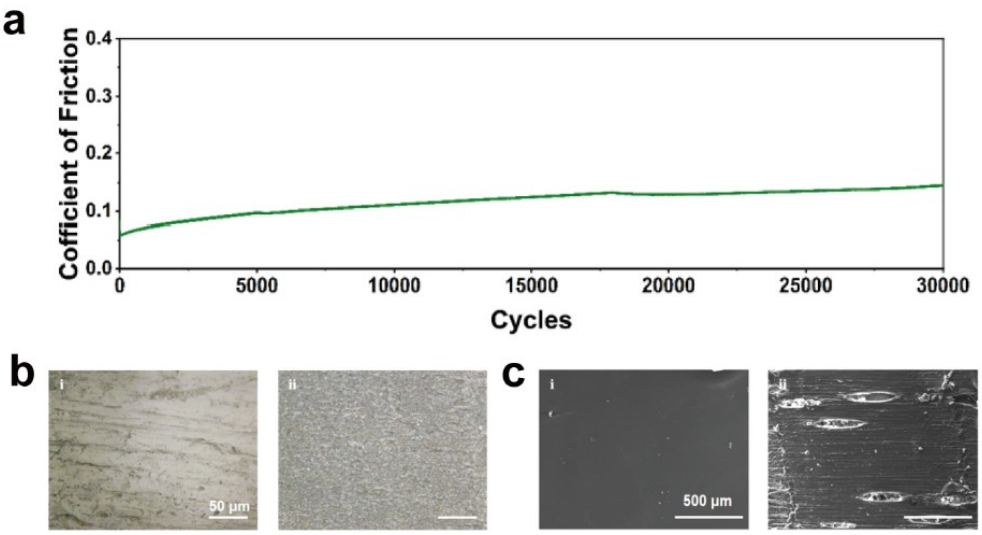

Fig. S5 (a) Dynamic COF of STOC hydrogel over 30,000 sliding cycles (PBS, 5 N, 1 Hz). (b) Optical microscopy images and (c) SEM images of the hydrogel surface (i) before and (ii) after the friction test.