

Supplementary Information

Zwitterionic polyurethane thin films enabling wet conformal adhesion to dynamic tissues

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1. Experimental section

1.1 Materials

Polypropylene glycol (PPG, M_n : 1000) was purchased from Adamas (Shanghai, China). Hexamethylene diisocyanate (HDI) was purchased from Meryer (Shanghai, China). *N,N*-Dimethylformamide (DMF; anhydrous grade, 99.8%), *N*-methyldiethanolamine (MDEA), 1,4-butanediol (BDO), dibutyltin dilaurate (DBTDL, 95%), dibutylamine (99%), polyvinyl alcohol (PVA, M_w : 146–186 kDa) were purchased from Aladdin (Shanghai, China). 1,3-Propanesultone (99%), agarose (1% gel $>1200 \text{ g}\cdot\text{cm}^{-2}$), methyl red, bromocresol green were purchased from Heowns (Tianjin, China). Anhydrous sodium carbonate, chloroform, concentrated sulfuric acid (98%), hydrochloric acid, hydrogen peroxide ($>30\%$), diethyl ether, toluene, ethanol were purchased from Jiangtian Chemical (Tianjin, China). PPG, MDEA, BDO were treated with vacuum dehydration at 100°C for 4 hours before use, while the rest of the reagents were used as received without purification. The BCA protein assay kit was purchased from Beyotime (Shanghai, China). Roswell Park Memorial Institute-1640 (RPMI-1640), trypsin-EDTA solution (0.25%, phenol red-free), cell counting kit-8 (CCK-8) were purchased from Beijing Solarbio Science & Technology Co., Ltd. In addition, sodium citrate anticoagulant rabbit blood was purchased from Nanjing Sbjbio Life Science Co., Ltd.

1.2 Synthesis of ZWPU

TAPU is prepared by condensation polymerization reaction. Firstly, PPG (5 g, 0.005 mol) was weighed and placed in a 50 mL three-necked flask, and 10 mL of DMF was added, and stirred evenly. Then, HDI (2.523 g, 0.015 mol) and DMF (10 mL) were added, and the reaction was carried out at 80°C for 1.5 hours. After that, MDEA (0.5960 g, 0.005 mol) and DBTDL (0.01 g) were added and the reaction was continued for another 1.5 hours to synthesize the prepolymer. The content of -NCO was determined by titration with the toluene-dibutylamine method, and the corresponding amount of BDO was added for chain extension. After 4 h of reaction, the mixture was cooled to room temperature to obtain TAPU. During the reaction, a nitrogen atmosphere was maintained. Then, different contents of 1,3-PS were added to the above solution and reacted at 50°C for another 12 hours to obtain ZWPU with different molar ratios to MDEA. Finally, the obtained products were precipitated in diethyl ether, washed twice with diethyl ether, and then dried in a vacuum drying oven at 50°C for 3 days to remove the residual organic solvents.

1.3 General characterization of ZWPU

The characteristic functional groups were characterized by Fourier Transform Infrared Spectrometer (FTIR, iS50 FT-IR) and X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific). ¹H NMR spectra were recorded by Bruker AVANCE III HD 400 MHz using deuterated chloroform as the solvent. The thermal stability of the chain segments was tested by a thermogravimetric analyzer (TG 209 F3 Tarsu) within the temperature range of 0–600°C and at a heating rate of 10 K·min⁻¹. The thermal properties were tested by differential scanning calorimetry (DSC 214 Polyma) within the temperature range of -70~220°C. After eliminating the thermal history, the cooling curve was obtained at a cooling rate of 20 K·min⁻¹ and the secondary heating curve was obtained at a heating rate of 10 K·min⁻¹. Throughout the process, nitrogen purge was maintained.

1.4 Preparation of ZWPU thin films

TAPU or ZWPU was dissolved in chloroform with different concentrations of 2 wt%, 3 wt%, and 4 wt%. First, a PVA aqueous solution (20 mg·mL⁻¹) was coated on the PET substrate using a 10 μm sized bar at a moving speed of 10 mm·s⁻¹, with PVA acting as a sacrificial layer. After the PVA layer was dried, wire bars with different bar sizes (6, 10, 20, and 40 μm) were employed to coat the TAPU or ZWPU solution. After the wet film was completely dried, the PET substrate with the PVA and the coating layers was cut to the suitable size (1×1 cm²) and

immersed in deionized water. After the PVA sacrificial layer was dissolved, the polyurethane film was released from the PET substrate and floated on the water surface. Then, the film was re-supported on a fresh silicon substrate for subsequent thickness measurement, morphological characterization, contact angle testing, and other analyses. The silicon substrate used in the tests were purchased from Lijing Electronics Co., Ltd. (Zhejiang, China), then the substrates were washed in a piranha solution composed of 98% sulfuric acid and 30% hydrogen peroxide (3:1, v/v), and finally rinsed in deionized water.

1.5 General characterization of ZWPU thin films

The thickness of the films was determined by scratching the sample with a scalpel and then measuring the lateral profile by using a stylus profilometer (AlphaStep D-300). The surface morphology of the films was characterized by SEM (Apreo S, FEI) after sputter coated with palladium. The hydrophilicity of the films before and after the modification of zwitterionic groups was evaluated by water contact angle measurement (JC2000FM, Shanghai Zhongchen Digital Technology Equipment Co., Ltd.). The films were re-supported on the quartz glass and the optical transparency of the films were evaluated by UV-visible spectrophotometer (Evolution 201, Thermo Fisher Scientific).

1.6 Antifouling performance test

The films were re-supported on the circular cover glass ($\phi 10$ mm, Shitai), and used after complete drying. To evaluate the anti-protein adsorption of the thin films, BSA, lysozyme and bovine fibrinogen were chosen as model proteins. TAPU and ZWPU films were immersed in solutions of BSA ($5 \text{ mg}\cdot\text{mL}^{-1}$), lysozyme ($5 \text{ mg}\cdot\text{mL}^{-1}$), and bovine fibrinogen ($3 \text{ mg}\cdot\text{mL}^{-1}$) respectively, and incubated at 37°C for 2 hours. After rinsed with PBS three times, the samples were exposed to a sodium dodecyl sulfate solution (SDS, 1 wt%), and ultrasonicated at room temperature for 30 minutes to detach the adsorbed protein. Then, according to the standardized procedure of the BCA assay kit, the absorbance was measured at 562 nm using a microplate reader (Infinite 200pro, Tecan). To assess platelet adhesion, the anticoagulated rabbit blood was first centrifuged at 1200 rpm for 15 min to obtain platelet-rich plasma (PRP). Then, 500 μL of PRP was covered onto the surface of TAPU and ZWPU thin films. The samples were incubated at 37°C for 2 hours and then gently rinsed three times using PBS. The lactate dehydrogenase (LDH) activity of each sample was measured according to the instructions of the LDH assay kit to quantify the platelet adhesion. To further observe the morphology of the

adherent platelets, all samples were fixed with glutaraldehyde solution (2.5 wt%) for 2 hours, then rinsed with deionized water to remove the glutaraldehyde, freeze-dried, and observed under SEM. In the cell adhesion experiment, TAPU and ZWPU thin films were sterilized by ultraviolet light and then placed in 48-well plate. Then $1 \times 10^4/200$ uL of L929 cell suspension was seeded on the surface of films and incubated at 37°C in a 5% CO₂ for 24 h. The cell culture medium solution was removed, and the samples were rinsed three times with sterile PBS solution. Finally, AO staining was used, and the cell adhesion was observed and recorded under a fluorescence microscope. Cell counts were conducted at three distinct locations for each sample.

1.7 Cytotoxicity test

After sterilization of TAPU and ZWPU thin films under ultraviolet light, the samples were immersed in RPMI-1640 medium containing 10% fetal bovine serum and 0.5% penicillin-streptomycin for 24 h to obtain their extracts. L929 cells (1×10^4 cells per well) were seeded in 96-well cell culture plates and incubated for 12 h. Then, the extracts were used to replace the RPMI-1640 complete medium. After further incubation for 24 h and 48 h respectively, the cytotoxicity of all samples was evaluated using the AO/PI staining kit and CCK-8 assay kit. Among them, the cell viability determined by the CCK-8 assay was calculated according to the equation: cell viability (%) = $(A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100\%$, where A_{sample} represents the absorbance of the CCK-8 solution after the cells were cultured in the sample extracts solution, A_{control} the absorbance of the CCK-8 solution after the cells were cultured in the complete medium, and A_{blank} the absorbance of the CCK-8 solution without cell culture.

1.8 Tensile test

A horizontal universal testing platform was self-assembled, consisting of a miniature load cell (ZNLBS-VI series S-type with a maximum of 1 N, Bengbu Zhongnuo Sensor Co., Ltd.), an electric sliding stage with a bidirectional screw, and two machined aluminum grips. A double-sided adhesive tape was shaped into rectangular frames of appropriate dimensions (outer: 3.0×2.5 cm², inner: 1.0×2.0 cm²). Before the test, we first adjusted the thickness of the thin films to keep it basically consistent. PET substrate with PVA and the coating layers was cut into 3.0×1.0 cm², then released in water and transferred to one side of the guide frame. All the samples were dried overnight at room temperature and tensile test was carried out on the following day. During the tensile test, we placed the guide frame with the adhesive side to

face down and contact with the aluminum grips, then used a soldering iron to melt the connections at the edge of the frame so that the mechanical sensor could accurately record the tensile reading. The effective dimensions of the samples were $1.0 \times 1.0 \text{ cm}^2$, and a crosshead speed was fixed at $3.2 \text{ mm} \cdot \text{min}^{-1}$, resulting in a strain rate of 0.0053 s^{-1} .

1.9 Wet friction behavior

Similar to the principle of a lap shear test, we measured the pulling force during the sliding of agarose gels on different thin film surfaces. We first used filter paper to absorb the water on the surface of the agarose gel. Then, varying amounts of water (0.005, 0.01, 0.02, and 0.03 mL) were introduced at the interface to mimic the wet environment. Agarose gels were prepared from 2 wt% and 5 wt% aqueous solutions and cut into cylinders with a diameter of 6 mm and a thickness of 4 mm. The films were cut into pieces of $6.0 \times 3.0 \text{ cm}^2$, re-supported on a cover slip, and allowed to dry completely before use. The testing platform was adapted from the above tensile test setup. A copper wire was used to connect the load cell and to horizontally pull the agarose gel at a speed of $1.6 \text{ mm} \cdot \text{min}^{-1}$, while the force was recorded in real time.

1.10 Cranial window for mouse brain bioimaging

Thy1-GFP mice over 8 weeks old were used for construction of brain edema model and in vivo imaging. Thy1-GFP mice express green fluorescent protein in a subset of layer 5 pyramidal cells, enabled us to achieve fluorescence images that were suitable for evaluating the differences in the structure of neurons, as well as an optically accessible depth for two-photon microscopy. First, the mice were anesthetized with tribromoethanol (Nanjing Aibei Biotechnology Co., Ltd.), and then the hair and skin were removed to expose the skull. The mouse was placed in a stereotaxic apparatus, and cranial skull with a large-area of $8 \times 8 \text{ mm}^2$ was removed to generate a cranial window using a microdrill (RWD Life Science, 78001) and forceps. Craniotomy was performed under a digital operating microscope (DOM-1001), with frequent washing using saline to remove bone debris. A piece of nonwoven fabric was used to transfer the ZWPU film onto the exposed brain tissue surface. The animal experiments were conducted in accordance with the animal experimentation regulations of Tianjin University and received approval from the Animal Ethics Committee of Tianjin University. The approval number for the animal experiments is TJUE-2022-054 (Approval of the Animal Ethical and Welfare of Tianjin University). Before two-photon imaging, the head plate was fixed to

the tissue and the thin film at the edge of the cranial window with dental cement (Nantong FeiYu biological technology Co., LTD.), and connected to the stage with screws. Image acquisition was obtained using a two-photon microscope (Olympus FVMPE-RS), and an XLPlan N 25×/1.05 W MP water-immersion objective lens ($\sim 509 \times 509 \mu\text{m}^2$). Image stacks were acquired with z steps of 1 μm and 512×512 pixels. The images were captured and processed with Olympus FluoView FV31S-SW and FV31S-DT software, respectively.

1.11 Wet conformal adhesion on rabbit lung

To evaluate the wet conformal adhesion performance on the surface of dynamic tissues under large-scale deformation, we designed a new experimental platform incorporating a fresh rabbit lung, a vacuum chamber, and a suction pump. Fresh rabbit lungs were obtained from a meat slaughterhouse, stored at 4°C, and used within three days. Polyurethane thin films coated on PET substrates were ink-printed with a square mesh (grid size: 2×2 mm²) using a laser printer (HP Color LaserJet Pro MFP M281fdw). First, PET substrate with PVA and the coating layers was cut into 2.0×2.0 cm², then released in water and transferred onto either side of the rabbit lung lobe with the help of a piece of nonwoven fabric. We placed the rabbit lungs covered with the thin films into a glass vacuum chamber (22×16×21 cm³) and used a suction pump to eliminate the air in the chamber. Then, the rabbit lungs started to expand. Once the pressure gauge reached its minimum (−0.1 MPa), we closed the vacuum valve and opened the exhaust valve. The rabbit lungs would then contract accordingly. This vacuum-exhaust cycle was repeated, and the deformation of the films was recorded and observed in real time through the transparent observation window.

1.12 90° peel test

For ease of observation, the film was first stained with Nile Red. The film was then cut into 1×1 cm² pieces and transferred onto the surface of pig skin using a nonwoven fabric. The pig skin was fixed, and a strip of high-adhesion tape was used to attach one end to the film and the other end to a fixture connected to the load cell. The peel test was performed at a peeling rate of 1.6 mm/min, and the corresponding force-displacement curves were recorded.

1.13 Subcutaneous implantation experiment

ZWPU thin films (8×8 mm²) were sterilized by ultraviolet irradiation for 12 hours. SD rats (220-230g) were anesthetized with isoflurane. The films were transferred from the water surface to the back of the rats using nonwoven fabric. One week later, the tissues in contact

with the materials on the back of the rats were collected, fixed with paraformaldehyde and embedded in paraffin. Then, the tissues were sectioned and stained with hematoxylin and eosin (H&E) to observe the inflammatory conditions of the tissues at the implantation site. The animal experiments were conducted in accordance with the animal experimentation regulations of Tianjin University and received approval from the Animal Ethics Committee of Tianjin University. The approval number for the animal experiments is TJUE-2022-054 (Approval of the Animal Ethical and Welfare of Tianjin University).

1.14 Data analysis

All experiments were performed at least three times ($n \geq 3$), and all data were presented as mean \pm standard deviation (mean \pm SD). Origin 2021 and ImageJ softwares were used for the data and image analysis.

2. Supporting figures

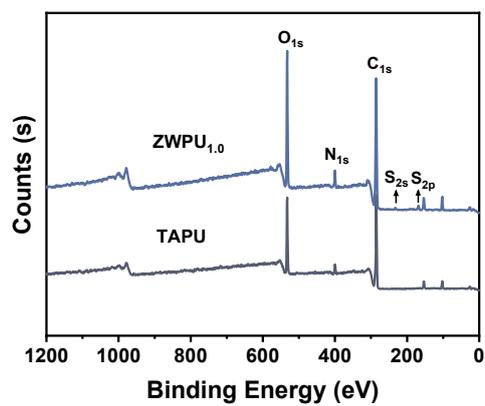


Figure S1. X-ray photoelectron spectroscopy survey spectrum.

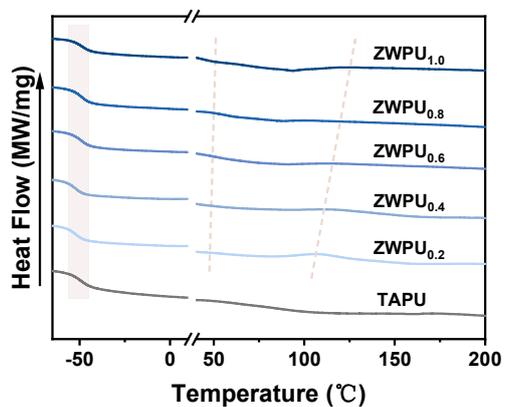


Figure S2. DSC spectra of TAPU and ZWPU.

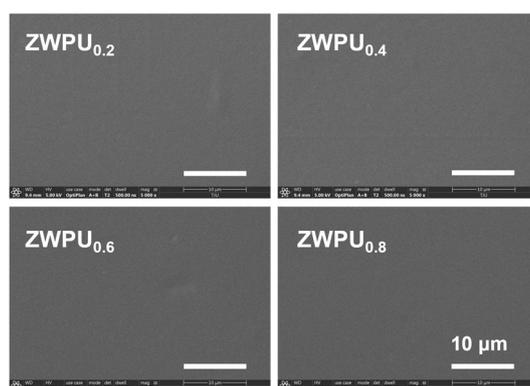


Figure S3. SEM images of ZWPU_{0.2}, ZWPU_{0.4}, ZWPU_{0.6}, ZWPU_{0.8} thin films surfaces.

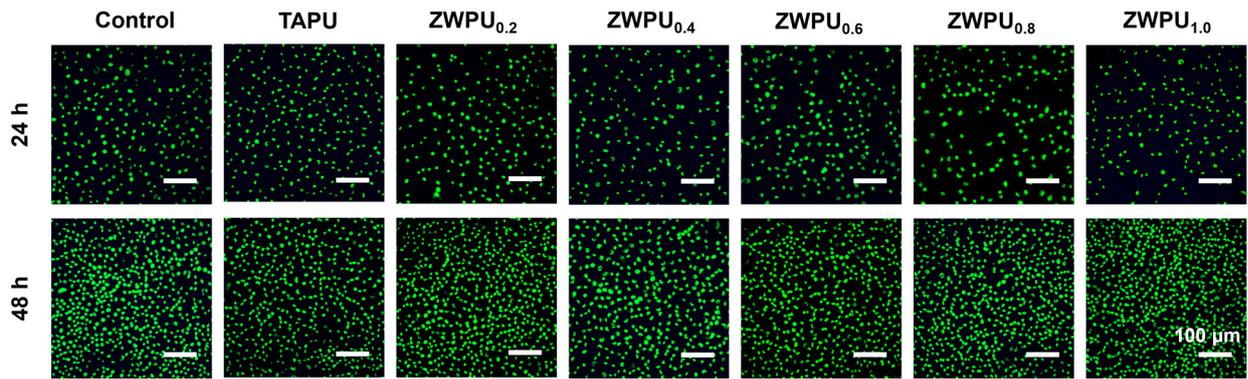


Figure S4. Fluorescence staining images of L929 cells in the cytotoxicity test. In the field of view, a large number of live cells (green) were observed. Compared with the fluorescence microscopic image after 24 h of culture, all groups of L929 cells showed a significant proliferation trend after 48 h of culture.

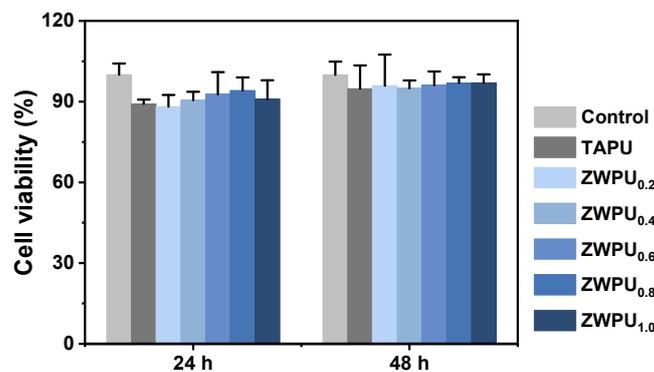


Figure S5. Cell viability determined by CCK-8 assay. The cell viability of the L929 cells in the control group (cultured in complete medium) was normalized to 100%, and the cell viability of the experimental groups was above 90% after 24 h, and above 95% after 48 h of culture. There was no significant difference between the groups, which indicates that the TAPU and ZWPU films have no cytotoxicity.

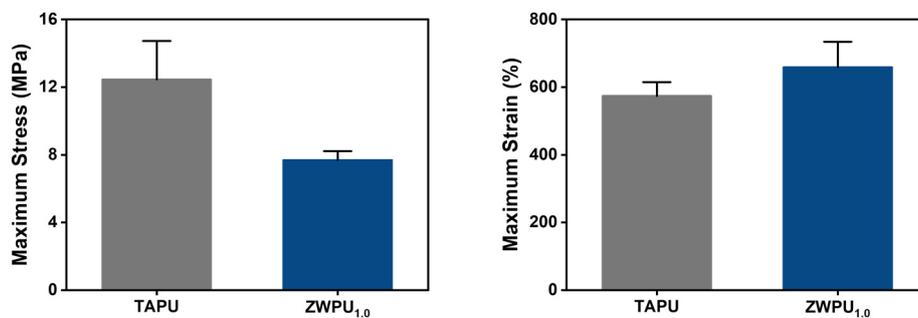


Figure S6. Average maximum stress and average maximum strain of TAPU and ZWPU_{1.0} films on stress-strain curves.

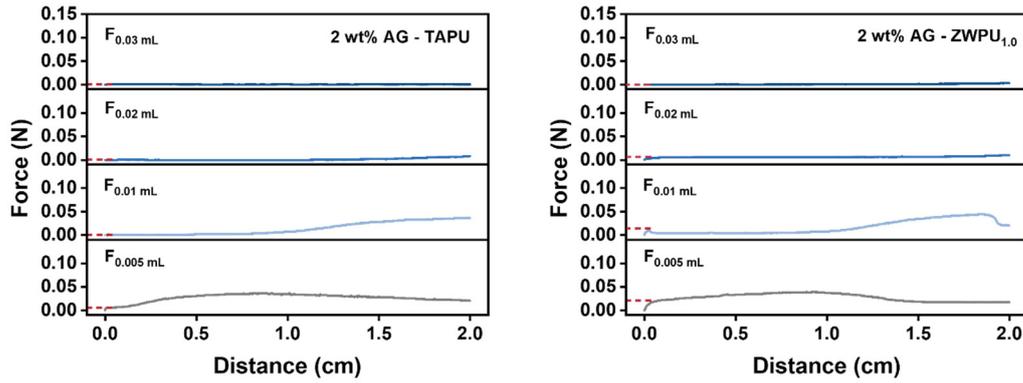


Figure S7. Recorded force while moving an agarose gel (2 wt%) on the surface of TAPU and ZWPU_{1.0} thin films with varying amounts of water at the interface.

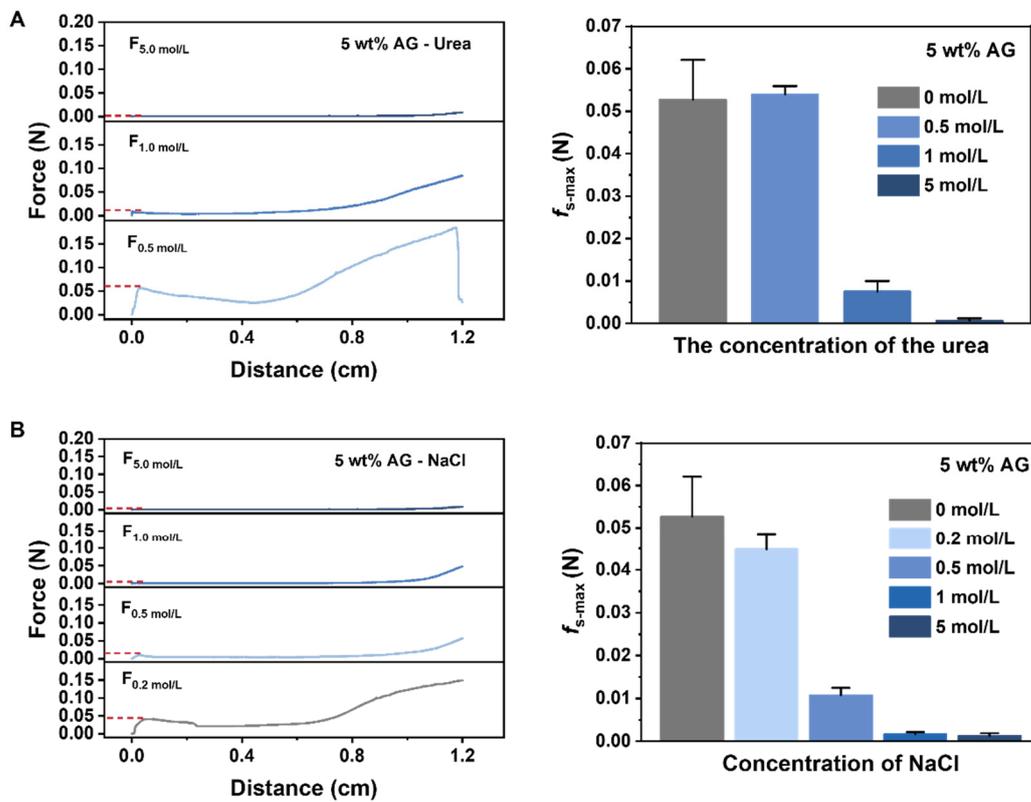


Figure S8. Recorded force while moving an agarose gel (5 wt%) on the surface of ZWPU_{1.0} thin films with different concentrations of urea (A) and NaCl (B) aqueous solutions (0.005 mL) at the interface.

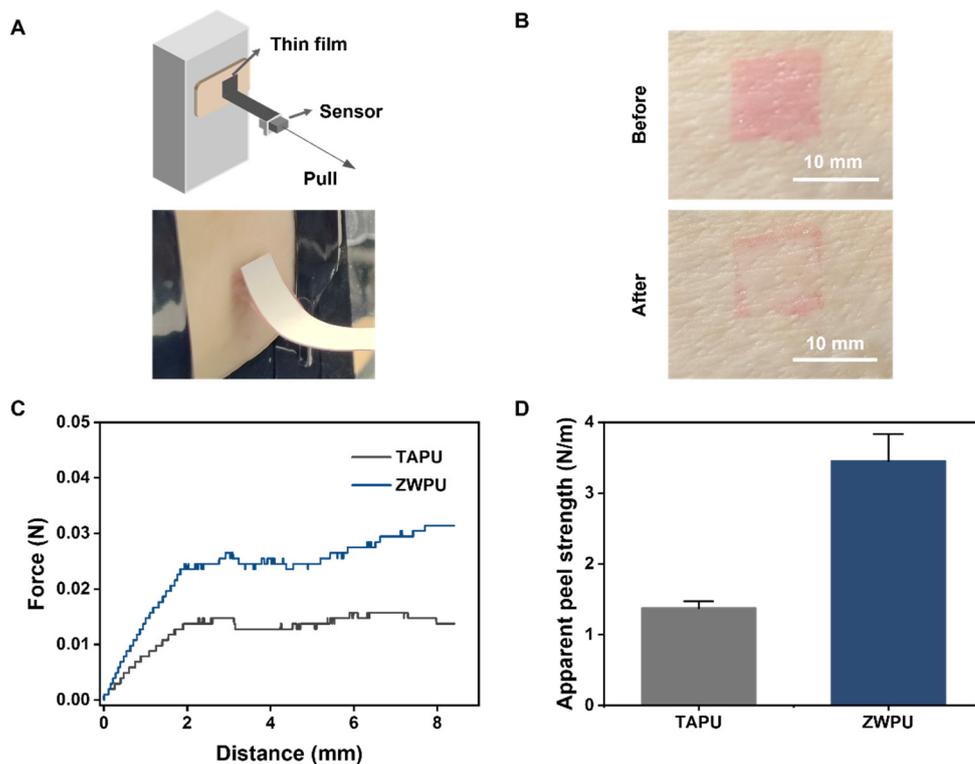


Figure S9. 90° peel test. A) Schematic diagram of the 90° film peel test and optical photos of the peeling process. B) Optical photos of the pig skin area covered by the thin film before and after the test. C) Representative force-displacement curves of ZWPU and TAPU films. D) Apparent peel strength.

Figure S9A shows the schematic diagram of the 90° film peel test and the representative photos during the peeling process. Figure S9B shows the representative photos of the pig skin area covered by the thin film before and after the peel test. It is clear that a portion of the film remains on the pig skin surface after the experiment, indicating that the internal continuity of the film is disrupted during the debonding process, and therefore the failure mode in this system is essentially a cohesive failure. The force-displacement curve during the peeling process is shown in Figure S9C, and the apparent peel strength is shown in Figure S9D. It must be noted that in our 90° film peel test, all measurements were terminated due to the occurrence of film rupture. Considering that the film is extremely thin and mechanically weak, it is stretched during debonding until fracture occurs. Therefore, the measured “maximum force” reflects a combined effect of the adhesion strength and the intrinsic strength of the film, and the measured apparent peel strength is much smaller than the real peel strength. Meanwhile, the measured peel force is of the same order of magnitude as that observed in the tensile test, which further supports this point. In fact, from the stress-strain curve, it can be seen that the mechanical strength of the ZWPU film is lower than that of the TAPU film.

However, the 90° film peel test shows that the apparent peel strength of the ZWPU film is greater than that of the TAPU film. This strongly proves that the adhesion strength of the ZWPU film on the tissue surface is higher than that of the TAPU film.

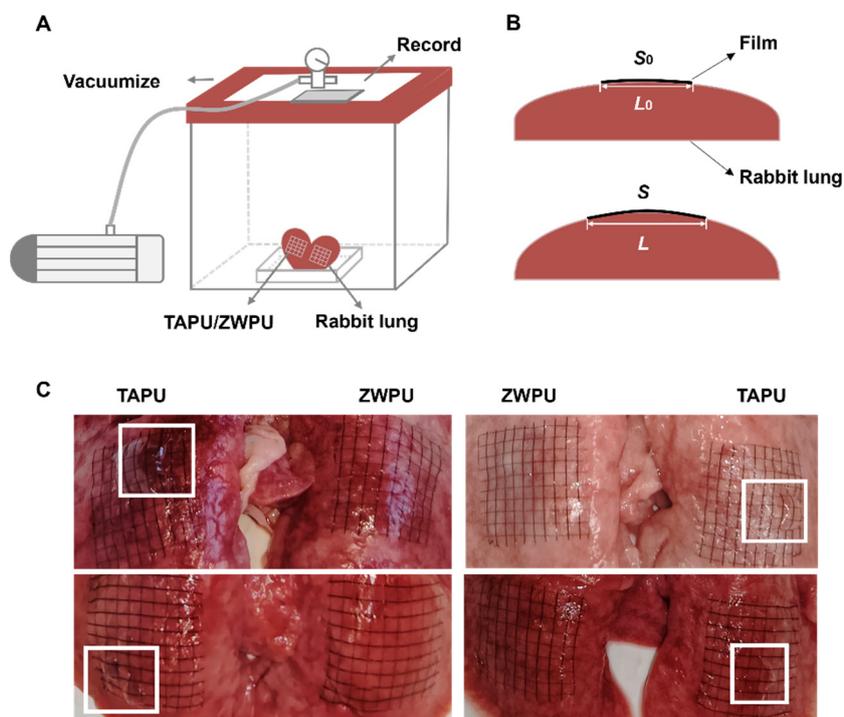


Figure S10. A) Schematic diagram of the rabbit lung conformal adhesion model test. B) The projected length ratio (L/L_0) and the stretch ratio (S/S_0). C) Air bubbles appeared beneath TAPU films in multiple experiments.

In materials mechanics, the stretch ratio provides a geometrically exact measure of deformation, whereas engineering strain is a linear approximation that is only valid in the small-deformation regime. To calculate the stretch ratio, we referred to the Digital Image Correlation (DIC) technique commonly used for strain analysis. Specifically, the films were ink-printed with a grid mesh. Since no stretching occurs in the film during the nonwoven fabric transfer process, the original arc length corresponds to the original grid line length ($S_0 = 2$ mm). The entire lung expansion process was recorded using an imaging acquisition system, and the grid line length (L) was measured during expansion. It should be noted that the grid line lengths measured in this work correspond to the horizontal projected lengths, rather than the actual arc lengths (S) on the expanded lung surface, as shown in Figure S10B. The characteristic size of the printed grid (2 mm) is much smaller than the radius of curvature of the rabbit lung surface (on the order of 10–30 mm). Under this small-length-scale condition,

the local lung surface can be approximated as planar, and the difference between the arc length and its horizontal projection is negligible. Therefore, we used the horizontal projected length ratio (L/L_0) to approximate the stretch ratio (S/S_0), which was defined as the ratio of the projected grid length after and before deformation and provides an operational and reliable measure of the local surface stretch.

The methodology for calculating the deformation degree of the thin films adhered to rabbit lungs is as follows: (1) The test schematic diagram is shown in Figure S10A. The starting and ending frame were selected from the recorded video, corresponding to the images of the rabbit lung before and after expansion, respectively, for subsequent analysis. (2) Since the film surface undergoes nonlinear deformation and the grid no longer retains a regular square shape, the grid-line lengths in the vertical and parallel directions were measured separately. Grid nodes were marked using ImageJ in the images before and after expansion, and the coordinates of each node were obtained, from which the horizontal projected length ratio (L/L_0) was calculated. (3) Due to the heterogeneity in mechanical properties and the complex geometry of different lung regions, lung expansion is highly nonlinear. To reduce measurement uncertainty, the area close to the observation window was selected as the region of interest (ROI, white box), the area with pronounced deformation at the center was defined as the center region (yellow box), and the remaining area was defined as the edge region. Eight measurements were randomly selected for each region, the highest and lowest values were discarded, and the average was calculated.

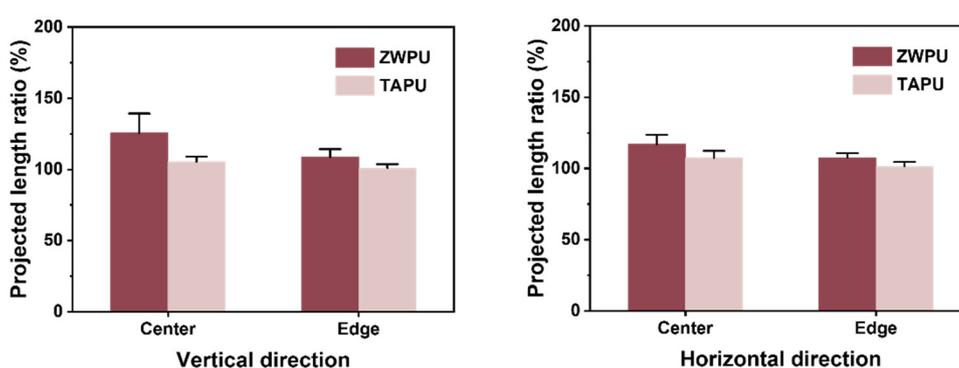


Figure S11. The projected length ratio of the film on different surfaces of rabbit lungs ($n=6$).

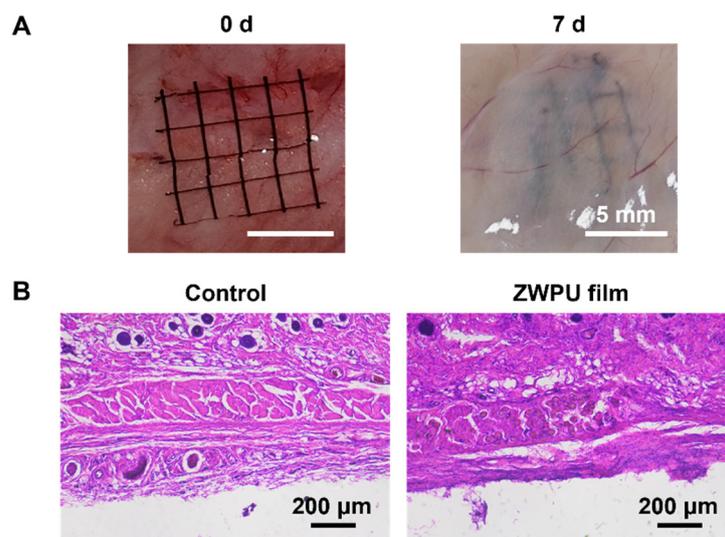


Figure S12. A) Optical images of the ZWPU film with a grid pattern during implantation and 7 days after implantation. B) H&E staining images (hematoxylin and eosin) of skin tissues from the blank control group and the group implanted with the ZWPU thin film.

We implanted the ZWPU film with grid pattern, after sterilization by ultraviolet irradiation, subcutaneously on the dorsal side of rats. Seven days after implantation, the ZWPU thin film remained clearly observable (Figure S12A), indicating that the ZWPU film can persist in vivo without obvious degradation over this period, which is consistent with our expectations. Polyurethane-based materials are known to exhibit negligible in vivo degradation, which has enabled the development of long-term stable implantable devices, such as artificial blood vessels and artificial heart valves. H&E staining (Figure S12B) showed that one week after implantation, there was minimal inflammatory cell infiltration at the implantation site, comparable to that observed in the blank control group, indicating good biocompatibility of the ZWPU thin film.

3. Supporting movies

Movie S1. The tensile process of a ca. 1200 nm thick freestanding ZWPU_{1.0} film on a self-assembled horizontal tensile platform. The shaking was caused by handheld smartphone recording, and the video is displayed at 12.5× real-time speed.

Movie S2. Transferring the ZWPU thin film to the 8×8 mm² craniotomy induced brain edema region in mouse with the help of a piece of nonwoven fabric. The video was captured from the display screen of the digital operating microscope.

Movie S3. In vivo two-photon imaging across all layers of the cerebral cortex in Thy1-GFP mouse via ZWPU thin film cranial window (*xy*: 509.117×509.117 μm²; *z*: 0–800 μm with 1 μm step). The video was produced using Olympus FluoView FV31S-DT software.

Movie S4. The rabbit lungs covered with polyurethane thin films were placed in a vacuum chamber and subjected to a pressure reduction process from atmospheric pressure to a vacuum level of –0.1 MPa, during which the lungs continuously expanded and the films deformed accordingly (left lobe: ZWPU; right lobe: TAPU). The video was recorded with a fixed-position video camera and played in real time.