Electronic Supplementary Information

Artificial spinal dura mater made of gelatin microfibers and bioadhensive for preventing cerebrospinal fluid leakage

Shengdong Sun[a,b], *Hao Luo*[c], *Yuanfei Wang*[a,d], *Yongming Xi*[c,]*, *Kuanjun Fang*[a,b,e,f,]*, *Tong Wu*[a,b,c,g,]*

[a]Shandong Key Laboratory of Medical and Health Textile Materials, College of Textiles and Clothing, Qingdao University, Qingdao, 266071, China

[b]Collaborative Innovation Center for Eco-Textiles of Shandong Province and the Ministry of Education, Qingdao University, Qingdao, 266071, China

[c]Affiliated Hospital of Qingdao University, Qingdao University, Qingdao 266071, China

[d]Qingdao Stomatological Hospital Affiliated to Qingdao University, Qingdao, China

[e]Laboratory for Manufacturing Low Carbon and Functionalized Textiles in the Universities of Shandong Province, Qingdao, 266071, China

[f]State Key Laboratory for Biofibers and Eco-textiles, 308 Ningxia Road, Qingdao 266071, China

[g]Institute of Neuroregeneration & Neurorehabilitation, Department of Pathophysiology, School of Basic Medicine, Qingdao University, Qingdao, 266071, China

*Corresponding authors: twu@qdu.edu.cn (T. Wu), kjfang@qdu.edu.cn (K. Fang), xym700118@163.com (Y. Xi).

1. Materials and Methods

1.1 Materials.

The following reagents were purchased from Maclean unless otherwise indicated. Type A pigskin gelatin (gel strength 300 Bloom) was purchased from Sigma-Aldrich. Glacial acetic acid (99.5 %). Polyethylene oxide (PEO) (average Mv ~ 1,000,000, powder). Glutaraldehyde (50 % in H_2O). 1-Ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (98.5 %). N-hydroxysuccinimide (NHS) (99 %). Isophorone Diisocyanate (IPDI) (99 %). Polyethylene glycol (PEG 800). Dibutyltin dilaurate (95 %). Glycerol (99 %). Dopamine hydrochloride (DOPA) (98 %). L-929 complete medium (FuHeng, Shanghai), CCK-8 kit (Dojido, Japan).

1.2 Fabrication of Gel/PEO microfibers by SBS.

A certain proportion of gelatin and PEO were mixed and stirred in 80 % acetic acid solution overnight to obtain a clarified liquid. The solution was transferred into a 10 ml plastic syringe with the tip of the needle extruded at a distance of 25 cm from the collector, and the spinning time was 6 h. The Gel and PEO ratios were Gel/PEO 50, Gel/PEO 25, Gel/PEO 20, and Gel/PEO 15, respectively. The air pressure was adjusted to be 0.06 MPa, and the propulsion speed was 2 ml/h.

1.3 Crosslinking of the Gel/PEO microfibers and preparation of pure Gel microfiber membrane.

The prepared fiber membrane, placed in an altar containing glutaraldehyde solution, was steam crosslinked for 6 h to obtain crosslinked fiber membrane G/P n fibers (n is the ratio of gelatin to PEO, n=50, 25, 20, 15). GP1 was placed in an aqueous solution containing NHS and EDC dissolved in the aqueous solution, where the molar ratio of EDC/NHS was 2:1. After 24 h, the sample was rinsed with PBS 3 times, and then vacuum-dried for spare parts to obtain the pure gelatin fiber membrane GP2. GEL/PEO 50 is treated with glutaraldehyde to obtain GP1.

1.4 Preparation of polyurethane adhesives capped with dopamine (DPU).

In the polyurethane adhesive designed by Su, the addition of dopamine significantly improved the adhesive effect in wet conditions.[1] Its adhesive mechanism is the

formation of hydrogen bonds between molecules, rather than through cross-linking agents. We designed a new adhesive based on this method. Specifically, DPU was prepared using an R-value (-NCO: -OH) with a molar ratio of 1.2. Under N_2 protection and in the presence of catalyst dibutyltin dilaurate, 0.1 mol of IPDI was slowly added dropwise to a flask containing 0.06 mol of PEG at 85 °C for 2 h. The temperature was lowered to 45 °C, and 0.02 mol of glycerol was added dropwise. Then, the temperature was raised to 55 °C and the reaction was carried out for 2 h, polyurethane (PU) was obtained. Finally, 0.01 mol of DOPA was added at 60 °C, and the reaction was carried out for 2 h to obtain DPU (Collaborative Innovation Center for Eco-Textiles of Shandong Province and the Ministry of Education, Qingdao University, Qingdao, 266071. S4).

1.5 Characterization.

After drying the fiber membrane, the surface structure was observed under a scanning electron microscopy (SEM, Hitachi Regulus XIV 8100). This analysis was conducted using by FTIR spectroscopy (on an IRTracer-100 with an attenuated total reflection (ATR) attachment). The spectral scanning range was 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. The surface elemental content and valence states of the films with different treatments were tested by X-ray photoelectron spectroscopy (XPS, Axis Supra+, Japan). Fiber crystallinity was characterized by X-ray diffraction (XRD, Smartlab SE). The substance content and thermal decomposition temperatures of the differently treated fiber membranes were tested by a thermogravimetric analyzer (TGA500, American).

The tensile strength was measured by universal testing machine (Instron 5967 machine). After measuring the length (L), width (W), and thickness (D) of the fiber membrane, the fiber membrane was fixed at both ends of the tensile testing machine, and the tensile (F) and displacement (S) data were recorded at a speed of 20 mm/min to measure the maximum tensile stress and the maximum tensile strain. The Young's modulus was also calculated.

The water absorption of the fibers was measured by the weighing method. Samples previously weighed in the dry state were immersed in PBS (Chembio) solution and incubated at room temperature. At the 24-hour time point, the excess solution was

wiped away with filter paper and the wet weight of the sample was measured. The water absorptivity of the sample was calculated using the equation.

$$Water\ absorptivity(\%) = \frac{Ws - Wd}{Wd} \times 100\%$$

（Where Ws is the aqueous mass of the sample and Wd is the dry mass of the sample.）

Bonding experiments using pigskin. The membrane was applied to the pigskin in contact with the adhesive layer and they were pressed at 50 kPa for 5 min to measure the length (L) and width (W) of the bonded portion of the patches, fixed to the ends of a tensile tester (Instron 5967 machine), and stretched at a rate of 50 mm/ min. The stretch (F) and displacement (S) were recorded.

1.6 Sealing performance test.

Fresh pig small intestines and sheep bones were purchased directly from the market and used for the investigation. Sheep dura mater and porcine small intestinal membrane were cut into 2 × 2 cm slices and fixed to a measuring device connected to a hydromanometer and a syringe. An incision of 5 mm in diameter was then made in the sheep dura mater and porcine small intestine membrane, respectively. Subsequently, adhesive was applied to the fibrous membrane to adhere it to the incision. PBS buffer was then injected into the device, and the peak pressure during the experiment was used as the burst pressure. Five samples per group were tested.[2]

After the removal of the vertebral plate from the sheep's spine, the catheter was inserted and secured between two adjacent nerve root outlets. A peristaltic pump (LEAD-FLUID BF-100F, China) was used to deliver artificial cerebrospinal fluid with added crystal violet at a rate of 0.2 mL/min (consistent with the average flow rate of cerebrospinal fluid), and the other end of the catheter was connected to the waste cylinder. A catheter was used above the dural defect to deliver a continuous drip of artificial cerebrospinal fluid at a rate of 0.2 mL/min.

Dural defects and repairs were performed according to methods similar to those used in the porcine small bowel model. The length of the defect was 5 mm, the patch size was 4 × 6 mm, and the patch was applied by applying 30 kPa pressure with the ends of blunt-nosed forceps to the patch at the area where the patch meets the dura mater for 5

min. The area around the incision was moistened with water at regular intervals, and the dura incision was continuously photographed with a camera.

1.7 Cell experiment.

After sterilizing the gelatin microfiber membrane and DPU materials, extracts of the materials were prepared by adding 12 mg materials in 1 mL culture medium and placed at 4 ℃ for 24 h. The exacts were filtered with a 0.2-μm filter before further use. The culture medium placed at 4 ℃ for 24 h was used as control. L929 cells were cultured with the exacts from different groups at a seeding density of 2000 cells per well under a condition of 37 ℃, 95 % humidity, and 5 % CO_2. CCK-8 kit was used to test the cell proliferation at 1, 2, and 3 days post culture.

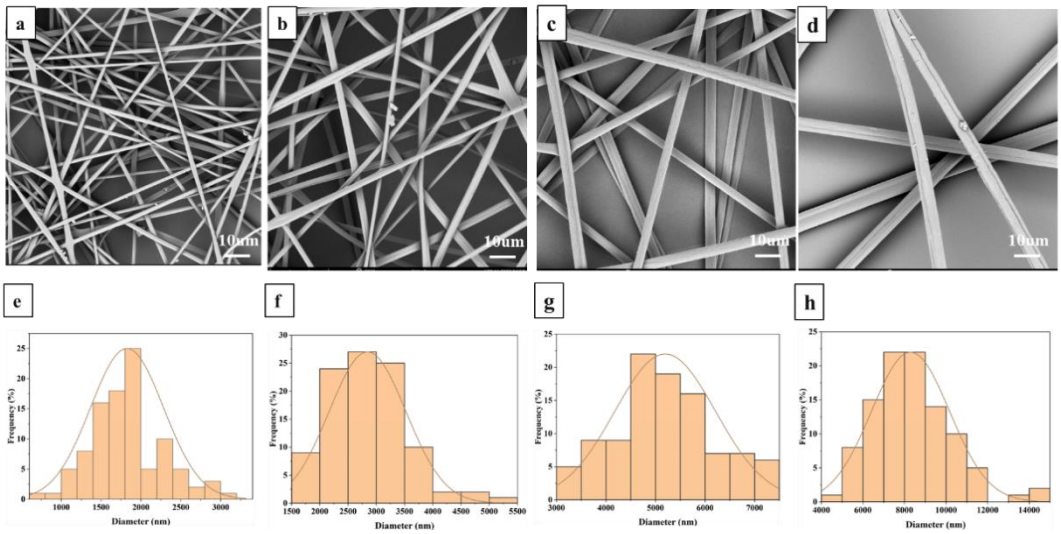


Fig. S1 SEM images showing the morphology of Gel/PEO n microfiber membrane. (a-d) Fiber morphology of Gel/PEO n. (e-h) Fiber diameters of Gel/PEO n (n is the ratio of gelatin to PEO, n=50, 25, 20, 15).

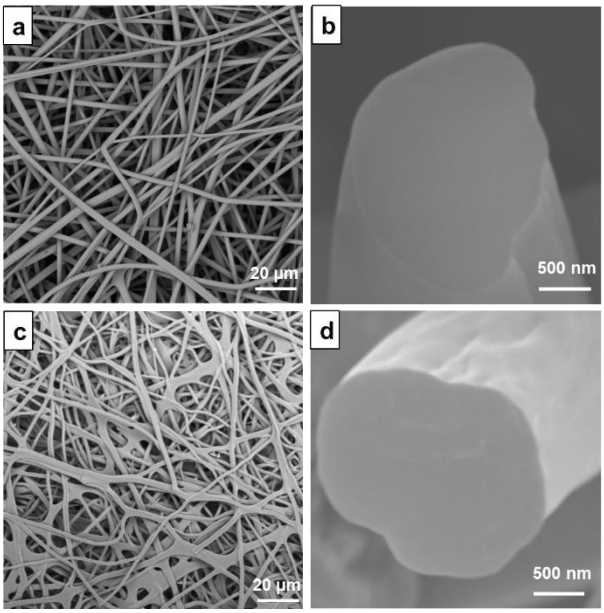
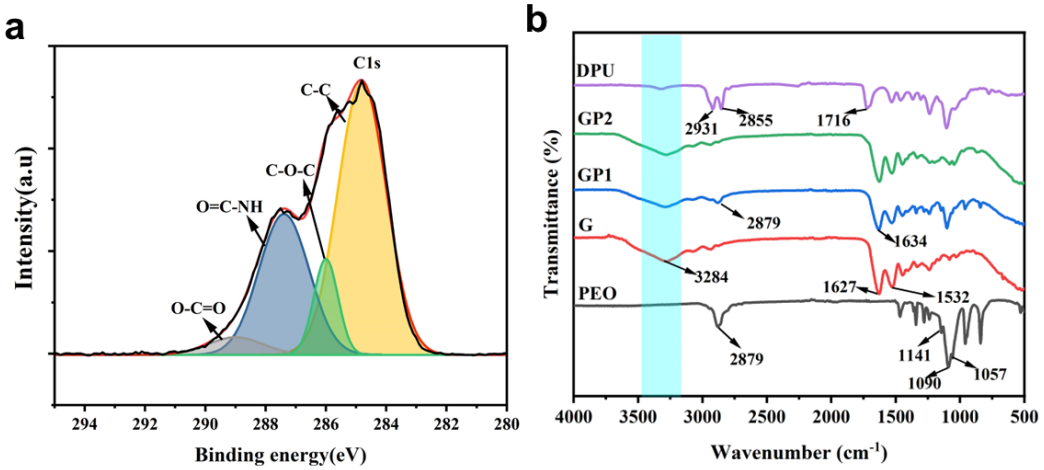


Fig. S2 SEM micrographs of the Gel microfiber membrane after different treatment of crosslinking. (a, b) SEM of G/P 50. (c, d) SEM of G/P 50-1 (G/P 50 crosslinked by EDC/NHS).



c

	C	N	O
G	65.93	15.27	18.8
GP1	69.17	10.81	20.03
GP2	64.85	15.25	19.89

Fig. S3 Chemical characterization of the fibers. (a) XPS C1s spectra of GP1. (b) FTIR test of the material. (c) Variation of elemental content on the surface of G, GP1 and GP2.

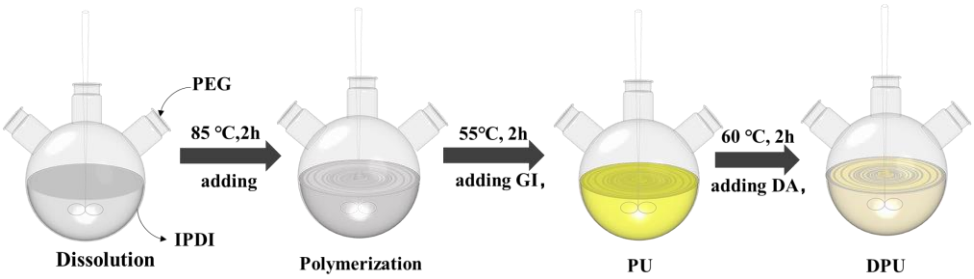


Fig. S4 Preparation process of the DPU.

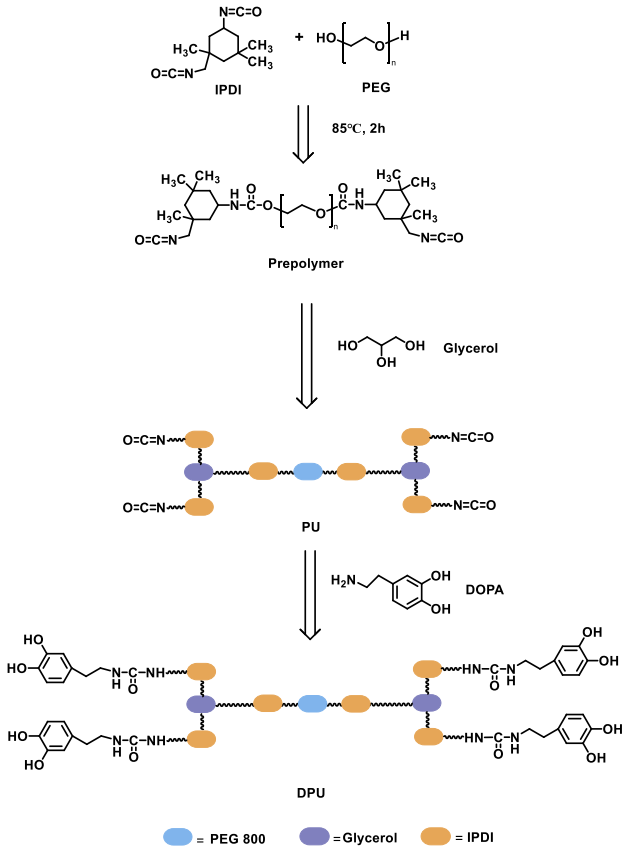


Fig. S5 Synthetic routes and chemical structures of DPU.

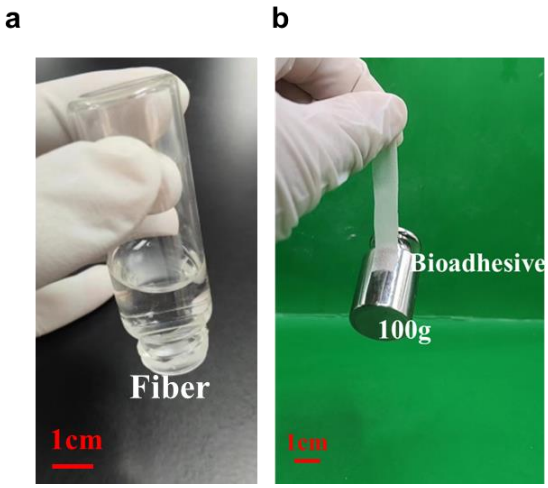


Fig. S6 Water sealing performance of GP2 fiber membrane and adhesion performance of DPU. (a) Physical diagram of the water sealing properties of the GP2 fiber membrane. (b) Bonding effect and mechanical strength of the fibers at 100 g.

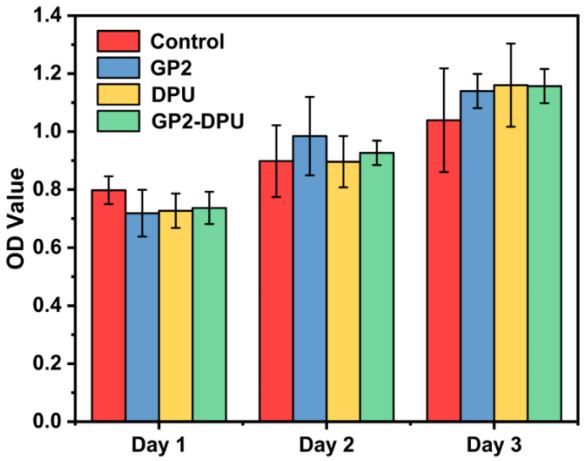


Fig. S7 Proliferation of L929 cells after culture with the extracts of different materials for 1, 2, and 3 days.

References:

1. T. Li, M. Sun and S. Wu, *Nanomaterials*, 2022, **12**, 784.

2. K. Deng, Y. Yang, Y. Ke, C. Luo, M. Liu, Y. Deng, Q. Tian, Y. Yuan, T. Yuan and T. Xu, *Neurol. Res*, 2017, **39**, 819-829.