Supporting Information

Fiber-shaped neural probe with alterable elastic moduli for direct implantation and stable electronics-brain interfaces

Chengqiang Tang[a], Songlin Xie[a], Mengying Wang[a], Jianyou Feng[a], Zhengqi Han[b], Xiaoying Wu[a], Liyuan Wang[a], Chuanrui Chen[a], Jiajia Wang[a], Liping Jiang[a], Peining Chen[a], Xuemei Sun[a], and Huisheng Peng[a]*

*[a]State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science and Laboratory of Advanced Materials, Fudan University, Shanghai 200438, China. *E-mail: sunxm@fudan.edu.cn.*
[b]School of Life Sciences, State Key Laboratory of Medical Neurobiology, Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200433, China.

Preparation of CNT fibers and insulation treatment. The CNT fibers were synthesized via floating catalyst chemical vapor deposition with thiophene (1-2 wt%) and ferrocene (1-2 wt%) as the catalyst, and flowing ethanol (> 97 wt%), Ar (200 sccm) and H2 (2000 sccm) as carbon source, carrier gas and reduction gas, respectively. The CNT aerogel was produced continuously at the hot zone of a tube furnace with the reaction temperature of 1200 °C, and it was then collected into cylindrical hollow socks. The CNT sock was pulled out of the furnace by a titanium rod and then densified through water and ethanol in turn. The CNT sock shrank immediately into CNT ribbon upon arriving at the water surface. It was finally washed by acetone for further densification, followed by drying, twisting, and collecting onto a spool to produce CNT fibers. By controlling the twisting and collection speed, we produced CNT fibers with designed specific diameters. The prepared CNT fibers (typical diameter of 20 μm) were cut into 12 cm long pieces and hung at both ends on a bracket. Then they were coated with parylene using a parylene deposition system coater (Specialty Coating System, PDS 2010 Labcoater). Name these fibers as I-CNTFs.

Structure characterization. Scanning electron microscope (SEM) images were obtained from Zeiss Gemini SEM500 FESEM, and photographs were taken from Olympus EX51. Raman spectra (**Fig. S2**) were acquired on a confocal Raman microscope (HORIBA XploRA) using a 50× objective and 532 nm laser.

Mechanical properties. For samples (Wet-MFNPs, brain) with a elastic modulus below 1 GPa, their moduli were measured with a bio-nanoindenter (Piuma Chiaro, Optics11, Amsterdam, the Neherlands). Note that the Wet-MFNP and brain tissue were measured in a liquid environment. For samples (Dry-MFNP, Au wire) with a elastic modulus larger than 1 GPa, their moduli were measured with a Bruker Hysitron Ti-950 nanoindenter equipped with a standard Berkovich probe. The stiffness of fibers wassted with dynamic mechanical analyzer (Q800, TA instruments). Samples of 1.2 cm in length were mounted with a single cantilever clamp. The sample were then tested with a frequency sweep (0.01-10 Hz) under controlled displacement (50 μm) at 37℃. The stress-strain curve was obtained by Instron 3365. Stretching speed was 5 mm/min.

Bending force of MFNP. First, we fixed the top end of a section of Dry-MFNP to the fixture of the tensile machine (Hengyi), and the length of the suspended portion was 5 mm. After immersion in artificial cerebrospinal fluid (Leagene Biotechnology) for

different times, the MFNP was pressed on the silicon slide to measure the force curve in the whole process. The loading velocity was 10 mm/min.

Adhesive force of MFNP. We added 0.6 g agarose (Sigma) to a glass bottle containing 99.4g of deionized water, covered the cap but did not tighten it, and kept it in a 110 ℃ oven for 1.5 h to obtain a clear solution. Then we poured the resulting solution into a mold and cooled it. A W wire (Worlwide Technology (S.H.) Co., Ltd.) and a Dry-MFNP were pierced into the same shaped agar gel, immediately pulled out and waited for 60 s (Dry-MFNP became Wet-MFNP), respectively, and the force curves were recorded. Both of them were penetrated to a depth of 5 mm.

Finite element simulation. We used COMSOL for finite element simulation analysis, the modulus of the brain tissue was set to 3 kPa, the modulus of the Dry-MFNP was set to 10 kPa, and the modulus of the W wire electrode was set to 200 GPa. The model dimensions are shown in **Fig. S11**. The two ends (red parts) of the medium were fixed, and a stick was utilized to press the middle part (red part) of the medium to reach the same deformation.

Electrochemical properties. We measured the probe impedance with an electrochemical workstation (CHI660E, CH Instruments Ins.) by using impedance function (**Fig. S14**). We measured cyclic voltammograms of the probe with an electrochemical workstation by using cyclic voltammetry function between the voltage limits of -0.8 and 0.8 V at a scan rate of 0.05 V/s (**Fig. S15**). For comparison, we used the same three-electrode method and tested them after immersed into artificial cerebrospinal fluid for 1 min. For verification of long-term stability, we tested the impedance once a week lasted for 4 weeks.

Swollen property and stability of Ca^{2+}crosslinked sodium alginate hydrogel. For swollen property, the MFNP were soaked in artificial cerebrospinal fluid at a constant temperature (37 ^{o}C), the diameter was observed and measured under the microscope (HORIBA XploRA). For stability, the MFNPs were soaked in artificial cerebrospinal fluid at a constant temperature (37 ^{o}C) and weighed the initial weight of each sample. After soaking for a certain period of time, they were took out and rinsed in deionized water, dried (80 ^{o}C, 2 h) and weighed.

Probe imaging in brain tissue. We imaged the probes in brain tissue by *in vivo* Micro-CT (SkyScan 1176, Bruker). First, the brain tissue without squeezing was imaged, then the compressed brain tissue was imaged, and finally their outlines were extracted.

***In vivo* electrophysiology.** All the animal experimental procedures were approved by the Ethics Committee of Fudan University and the International ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals were strictly followed. MFNP probes were tested in male mice (ICR, 6 weeks old, Shanghai SLAC Laboratory Animal Co., Ltd.) housed at ordinary animal room (12 h light/dark cycle, 22 ℃, food and water ad libitum). No animals were excluded from the analysis.

We performed surgeries on deeply anesthetized mice (2% isoflurane mixed in oxygen) positioned in a stereotactic frame (RWD). Cefuroxime sodium (2 mg/kg) was administered subcutaneously over the shoulders to reduce the inflammatory response, followed by the subcutaneous administration of 0.5% lidocaine directly under the scalp incision site. Then the scalp was removed by scissors. An electric drill was used to remove skull of implantation sites. One flat head screw was screwed to another side as ground and reference. All surgical instruments were sterilized.

In order to facilitate the clamping of the probe, we used silicone rubber to fix the top half of the probe on the polyethylene terephthalate (PET) plate. The exposed probe had a length of 5 mm. The bottom end of the probe was connected with a Cu wire by silver glue, and the other end of the Cu wire was soldered to the amplifier chip interface and then connected with Blackrock Microsystems. We loaded the PET plate into an FHC hydraulic micropositioner (FHC INC). Data analysis was performed with software spike 2 (CED).

For chronic recording, after the probes were implanted as described above, bare brain tissue was covered with a layer of agar gel (1 wt %), then with a layer of paraffin, and finally fixed to the skull with a dental resin adhesive (Super Bond C&B, SUN MEDICAL).

Immunofluorescence staining. To compare long-term tissue responses, MFNPs were implanted into the brain of mice, and nothing was implanted into the contralateral

hemispheres. One week after implantation, the mice were euthanized and the brain was carefully removed. The brain was cryoprotected in 4% paraformaldehyde solution overnight, and we then removed the tissue from the fixative in the fume hood, leveled the tissue of the target with a scalpel, and placed the trimmed tissue and corresponding label in the dehydration box. The dehydration box was placed in a hanging basket and dehydrated in a JJ-12J dewatering machine (Wuhan Junjie Electronics Co.,Ltd.) with a gradient of alcohol, alcohol benzene, xylene and paraffin. The paraffin-impregnated tissue was embedded in an JB-P5 embedding machine (Wuhan Junjie Electronics Co., Ltd.). The melted paraffin was first placed in the embedding frame. The tissue was taken out of the dehydration box before the wax was solidified, and it was then placed in the embedding frame according to the requirements of the embedding surface. After cooling at -20 °C, the paraffin wax was solidified, and the paraffin block was removed from the embedding frame and trimmed. The trimmed paraffin block was placed in a RM2016 paraffin slicer (Leica) and sliced to a thickness of 3 μm. The slices were floated on a spreader at 40 °C. The tissue was flattened on a warm water, the slides were picked up, and the slices were baked in a 60 °C oven. After the water was baked and the wax was roasted, the slices were taken out at room temperature for storage. (i) NeuN+GFAP+DAPI, the sections were sequentially placed in xylene I (15 min), xylene II (15 min), anhydrous ethanol I (5 min), anhydrous ethanol II (5 min), 85% alcohol (5 min), 75% alcohol (5 min) and distilled water. The resulting tissue sections were placed in a microwave oven for antigen retrieval in a repair kit filled with citric acid (pH 6.0) antigen retrieval solution (Servicebio). During this process, the buffer should be prevented from excessive evaporation and should not be dried. After cooling naturally, the slides were placed in PBS (pH 7.4) and washed three times for 5 min each time on a decolorizing shaker. We sliced the tissue slightly and dried it with a tissue pen (to prevent antibodies from flowing away). We then added BSA to the circle and incubate it for 30 min. The blocking solution was gently rubbed off, the primary antibody (NeuN, 1:8000; GFAP, 1:500) was dosed with PBS on the sections, and the sections were placed in a wet box and incubated at 4 °C overnight. A small amount of water was added to the wet box to prevent evaporation of the antibody. The slides were placed in PBS (pH 7.4) and washed 3 times on a decolorizing shaker for 5 min each time. After the sections were slightly dried, the secondary antibodies (HRP-labeled Goat Anti-Rabbit IgG (H+L), 1:500; Cy3 conjugated Goat Anti-rabbit IgG (H+L), 1:300; Servicebio) of the corresponding species of the primary antibody were added dropwise in the circle, and they incubated at room temperature for 50 min in the dark. After the

slices were slightly dried, we added autofluorescence quencher in the circle for 5 min, and rinsed them with running water for 20 min. After the slices were slightly dried, DAPI (4'6-diamidino-2-phenylindole) stain was added dropwise in the circle and incubated for 10 min at room temperature. The slides were placed in PBS (pH 7.4) and washed 3 times on a decolorizing shaker for 5 min each time. Slices were dried slightly and then sealed with anti-fluorescence quenching tablets. Confocal fluorescene imaging of the slices was acquired on a C2+ confocal microscope (Nikon). (ii) NeuN+Iba-1+DAPI, primary antibody was replaced with Iba-1, and the other steps were the same as (i).

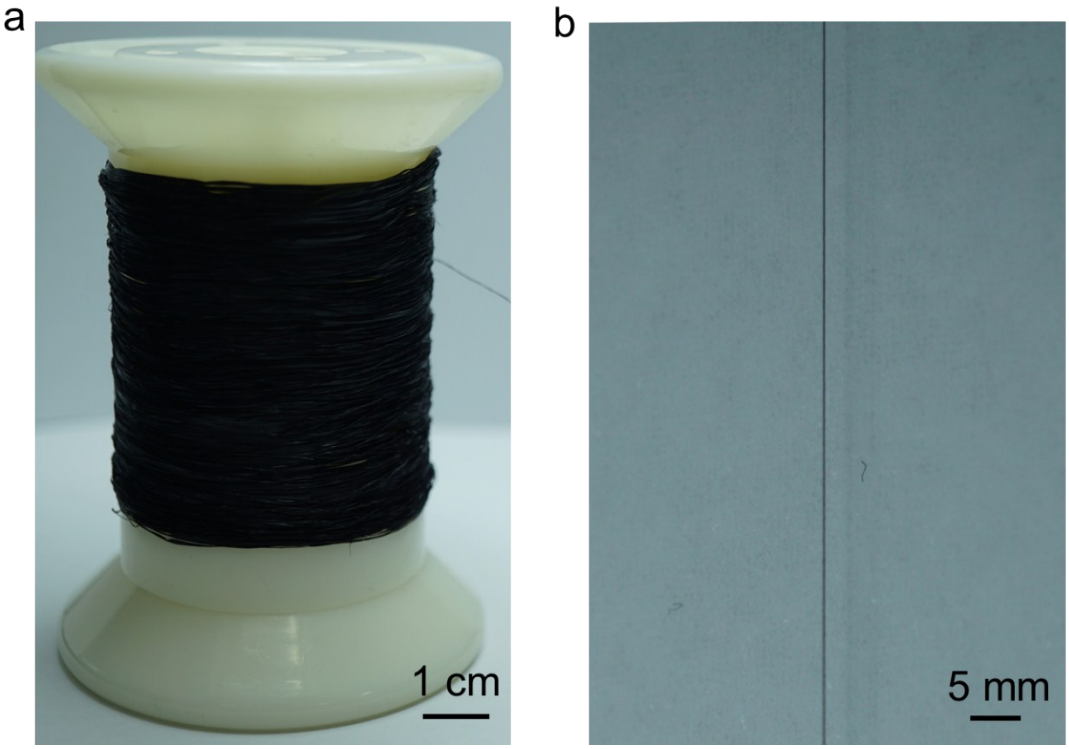

Figure S1. a, Photograph of a roll of the CNT fiber. **b,** A twisted CNT fiber.

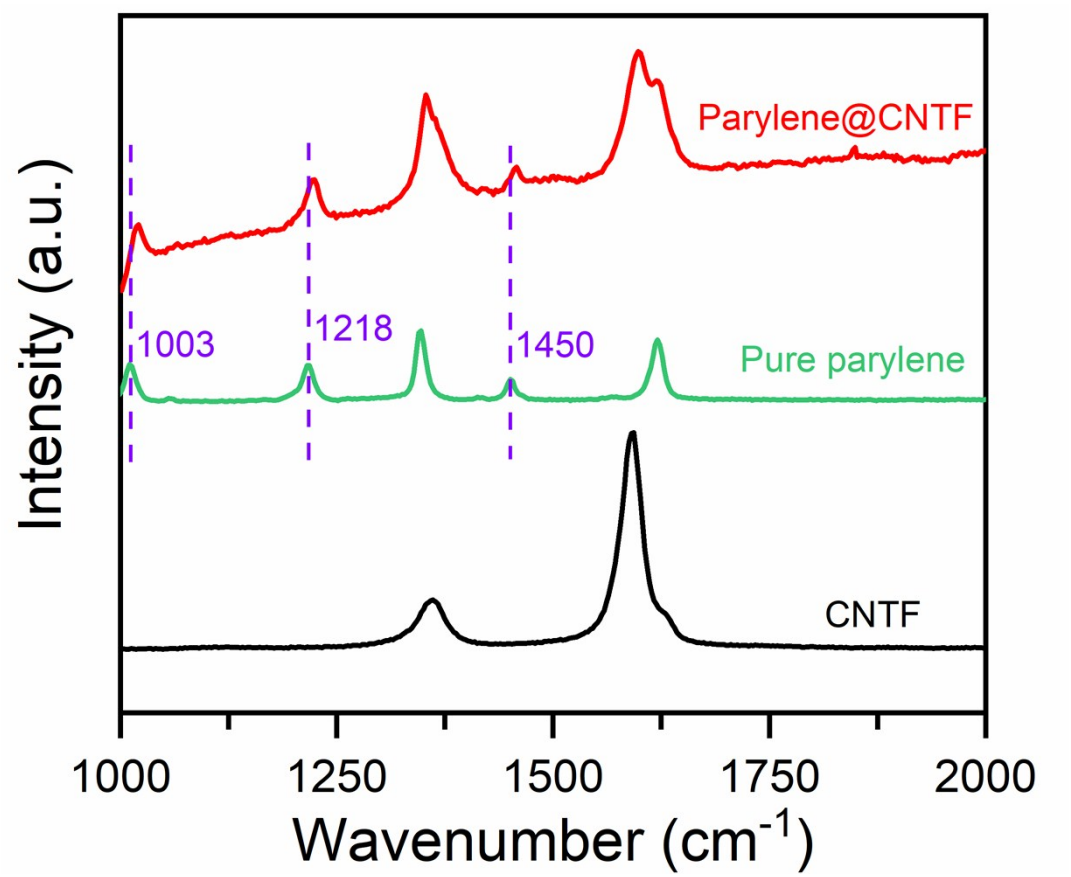

Figure S2. Raman spectra of the CNTF, parylene and CNTF coated with parylene on the surface.

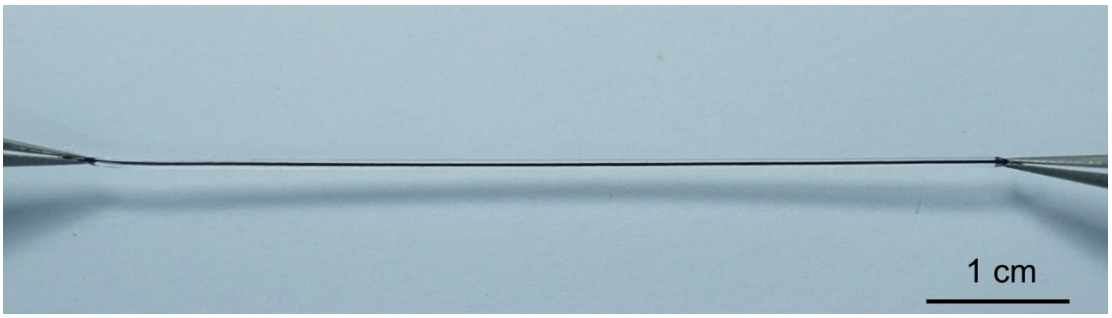

Figure S3. Photograph of a Wet-MFNP.

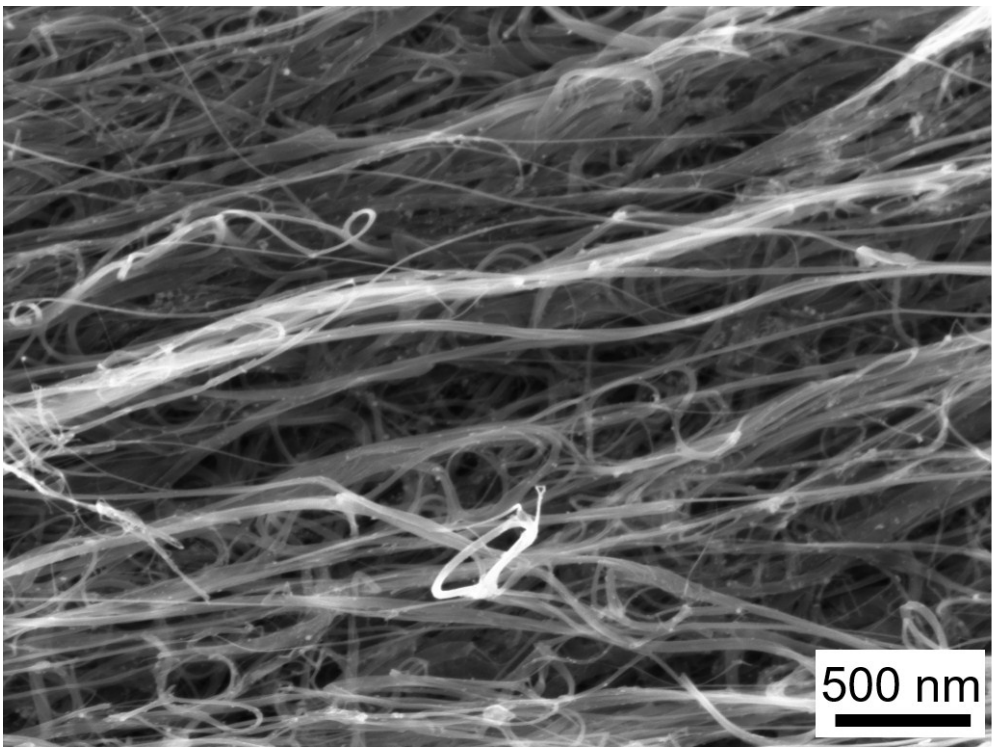

Figure S4. SEM image of a CNTF.

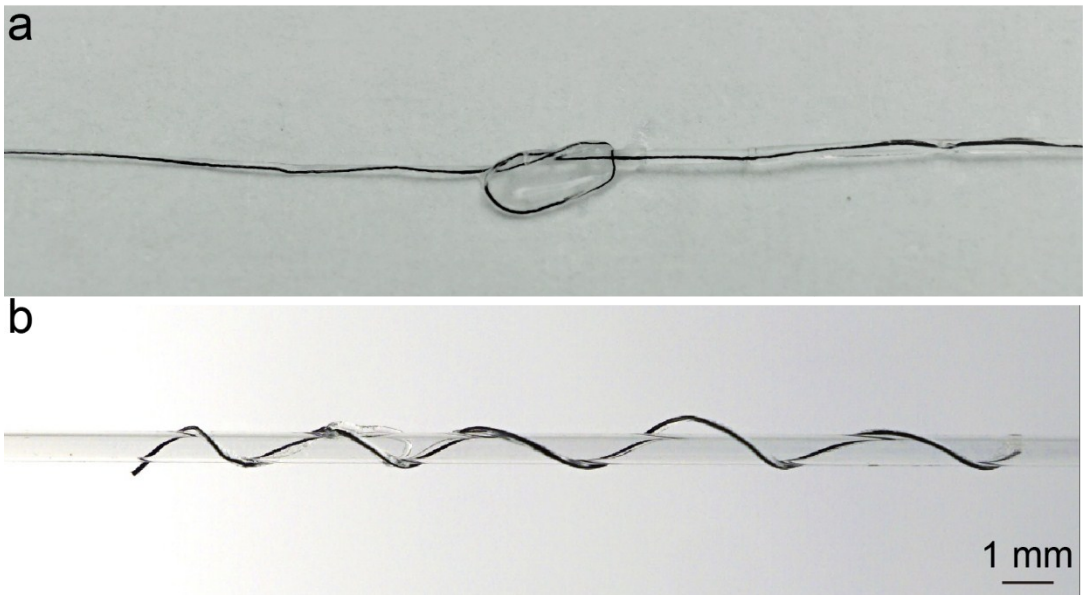

Figure S5. Photographs of the Wet-MFNP being (**a**) folded and (**b**) wrapped around a capillary.

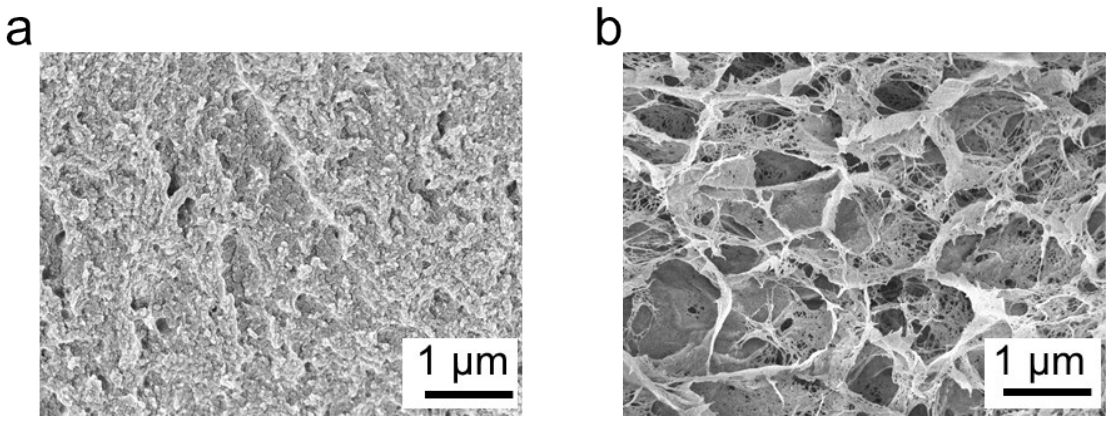

Figure S6. Scanning electron microscope (SEM) images of the internal structure of (**a**) a direct dry fiberand and (**b**) a wet fiber by freeze drying.

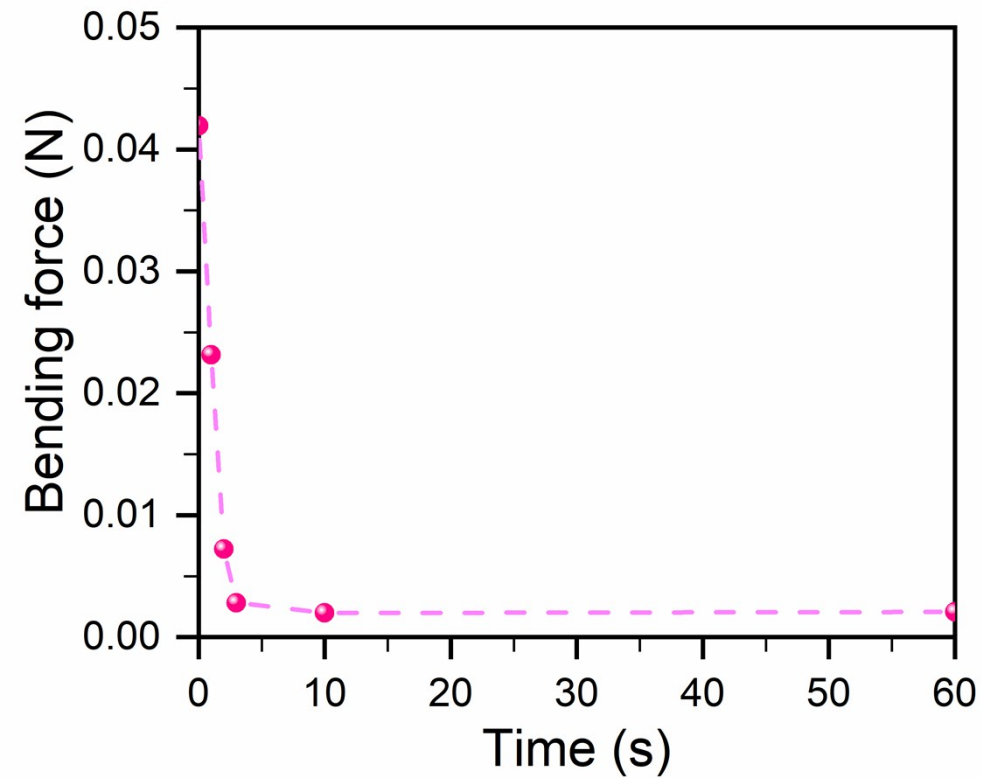

Figure S7. The tested bending force of Dry-MFNPs after immersed in artificial cerebrospinal fluid of mice for different periods.

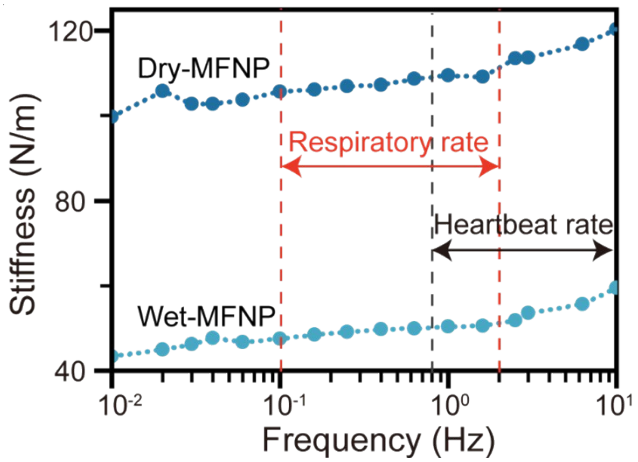

Figure S8. Measured bending stiffness of a Wet-MFNP and a Dry-MFNP at different frequencies with respiration and heartbeat frequencies covered in the testing range. The displacement amplitude was set as 50 μm.

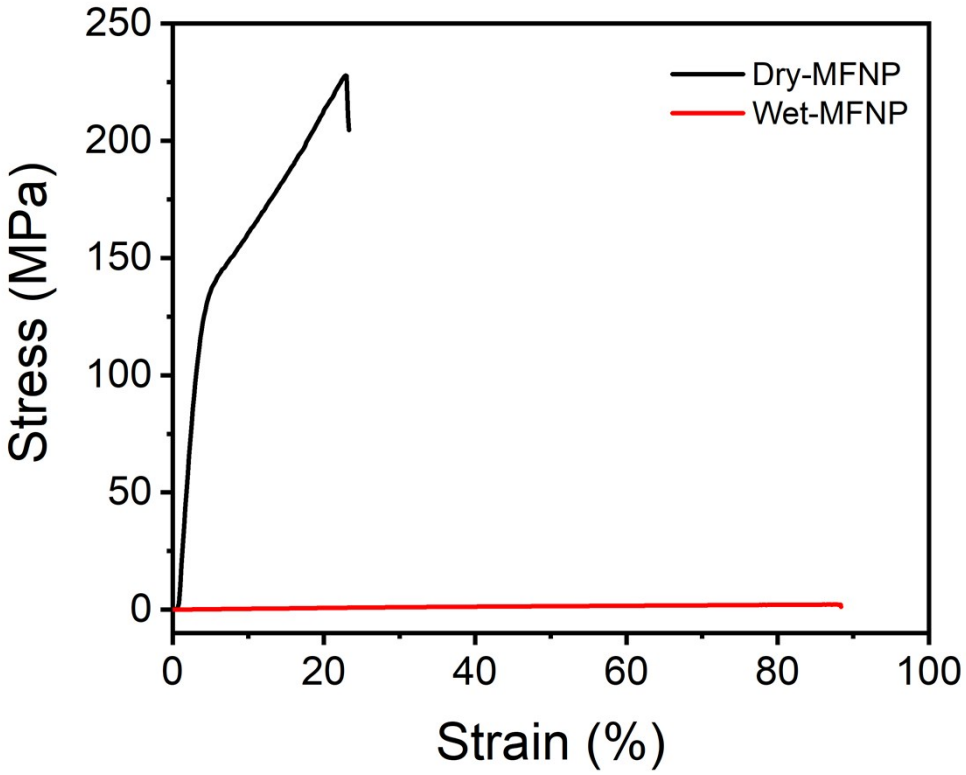

Figure S9. Stress-strain curves of dry and wet fibers.

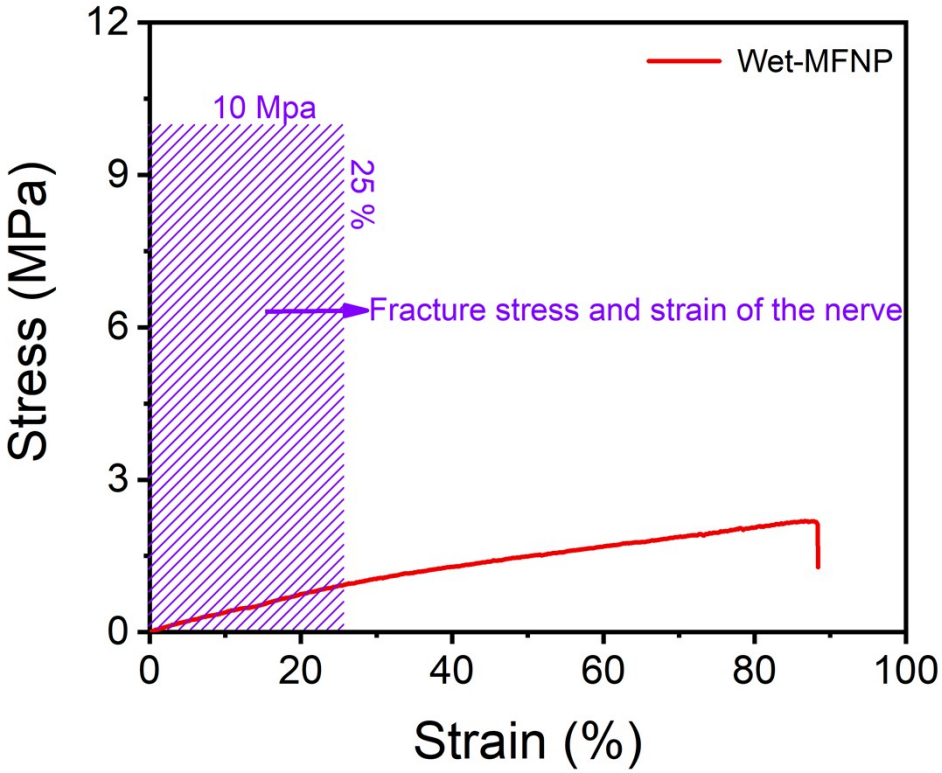

Figure S10 Stress-strain curve of the wet fiber and fracture stress and strain of the nerve.

Figure S11. Model and size for finite element analysis.

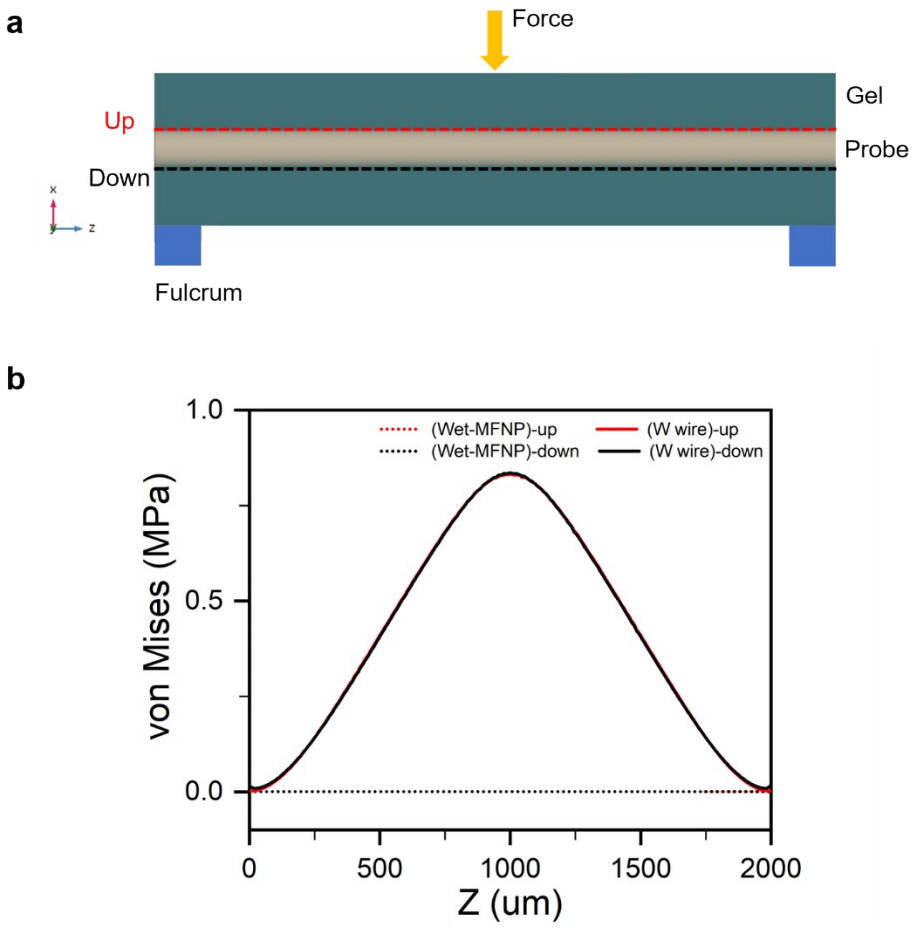

Figure S12. a, The gel was fixed at both ends, and an equal amount of displacement occurs in the middle. **b,** Distribution of von Mises in gel/probe interface.

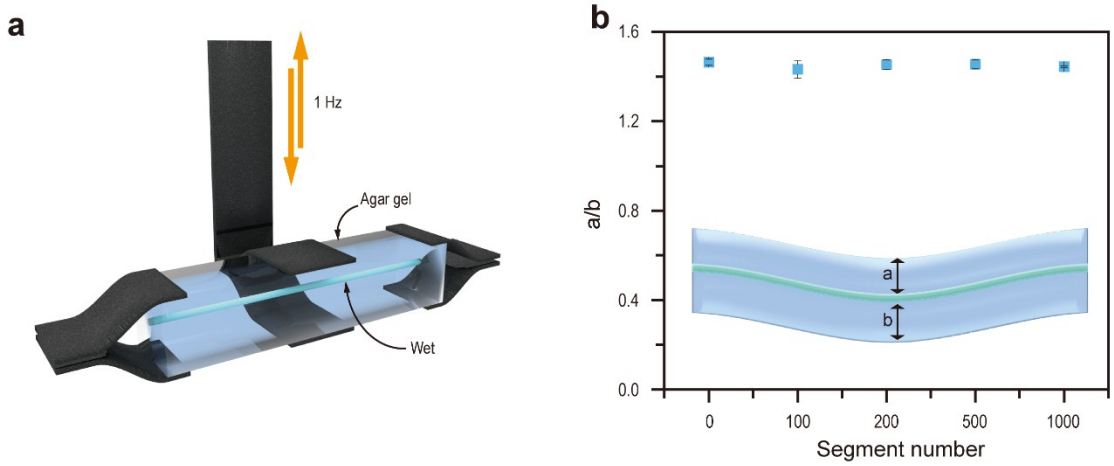

Figure S13. The schematic (**a**) and result (**b**) of cyclic deformation test of a Wet-MFNP in agar gel.

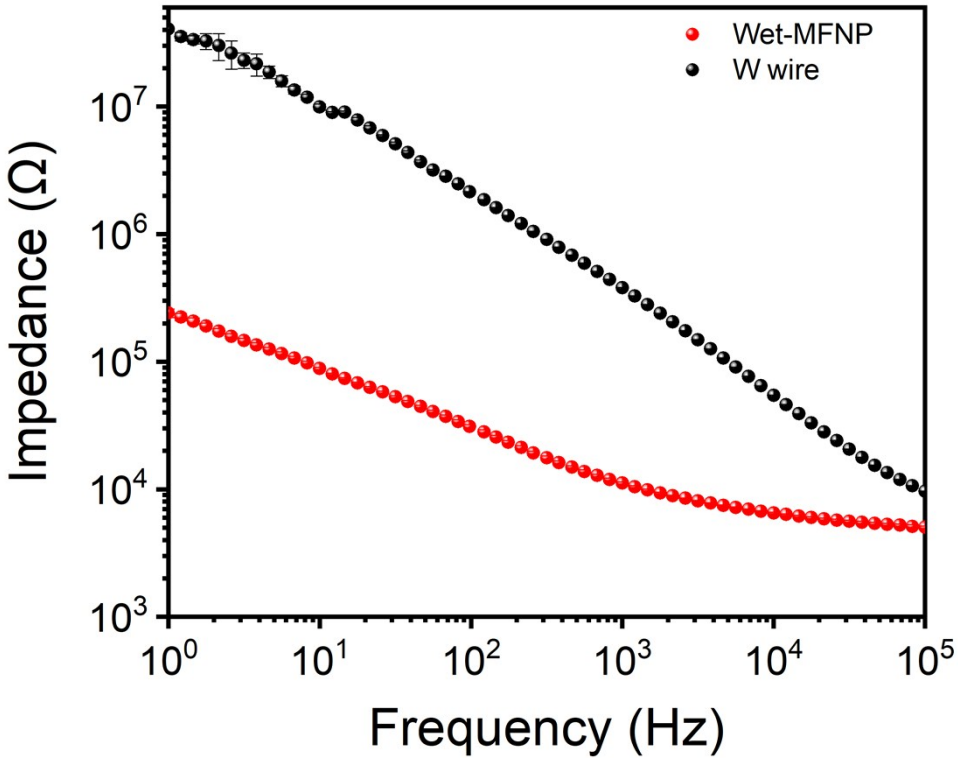

Figure S14. Impedance spectra of the Wet-MFNP and W wire.

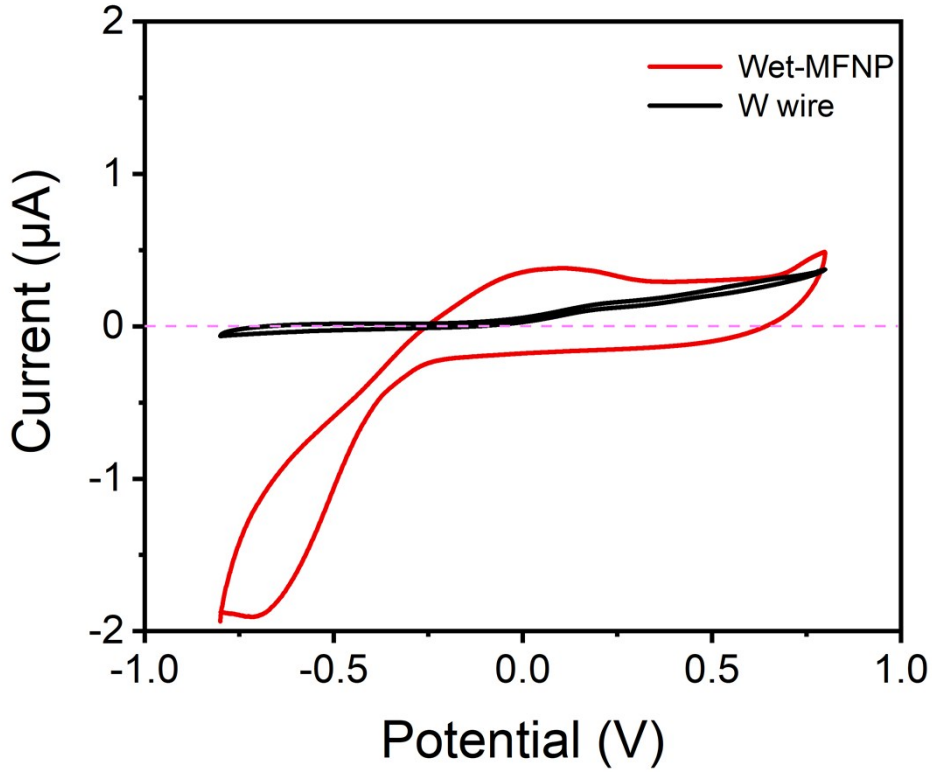

Figure S15. Cyclic voltammograms of the Wet-MFNP and W wire.

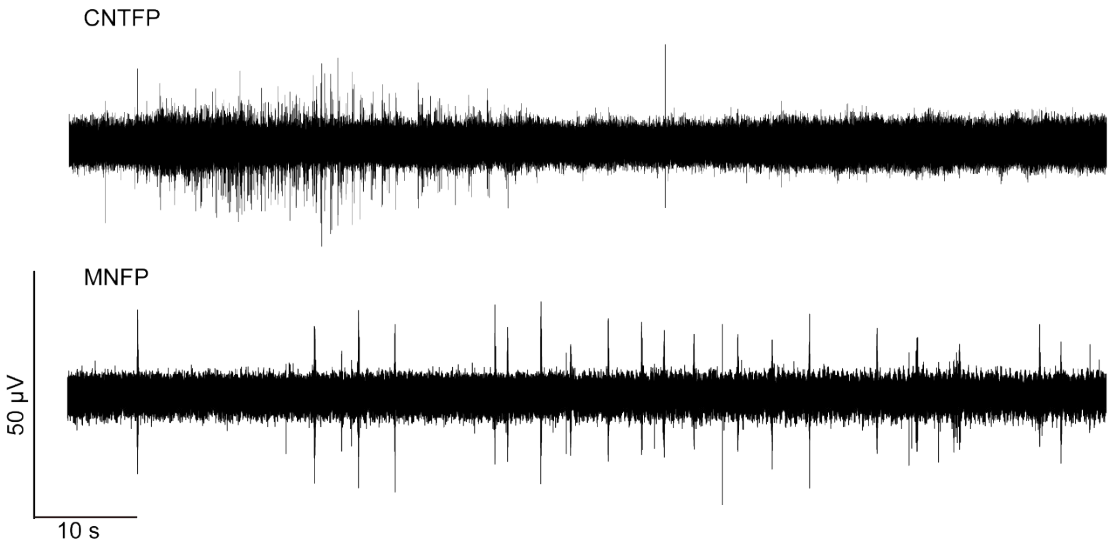

Figure S16. Neural signal recording of an I-CNTF and MFNP *in vivo.*

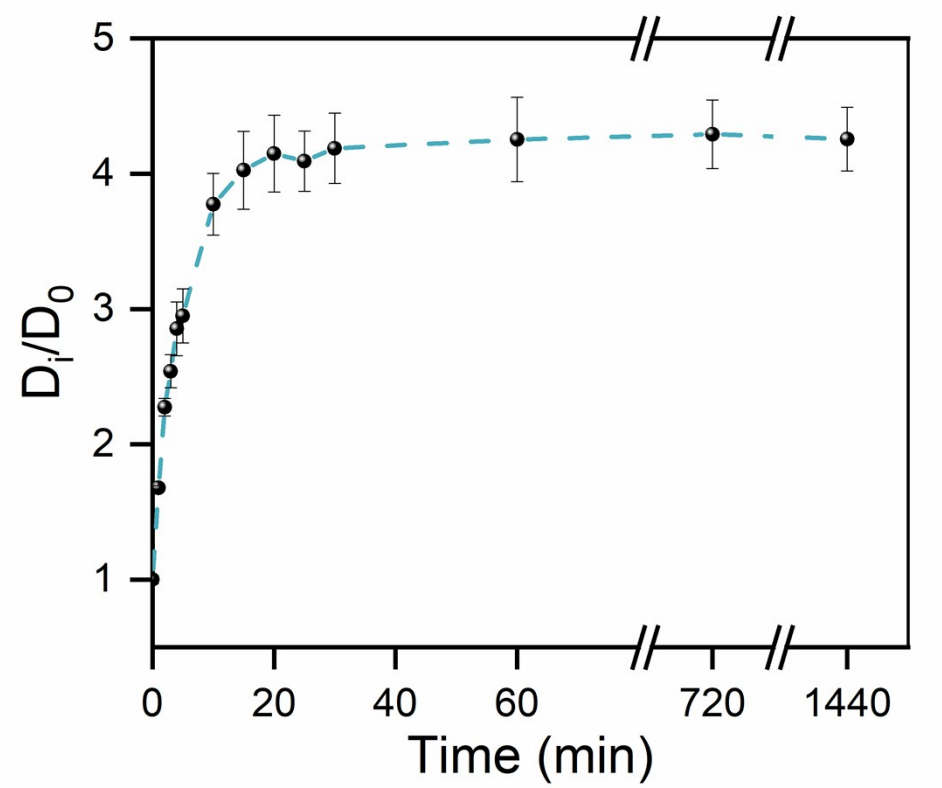

Figure S17. Swollen property of the MFNP i*n vitro* (D_0: initial diameter; D_i: diameter after i min).

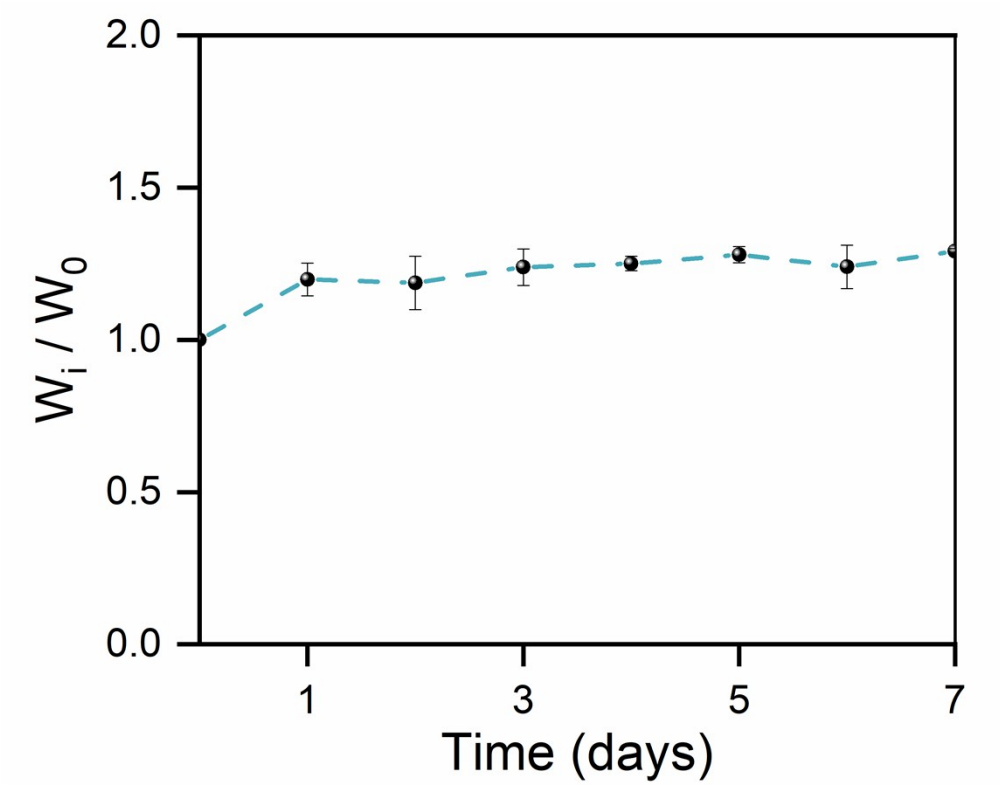

Figure S18. Stability of Ca^{2+}crosslinked sodium alginate hydrogel layer *in vitro* (W_0: initial weight; W_i: weight after i days).

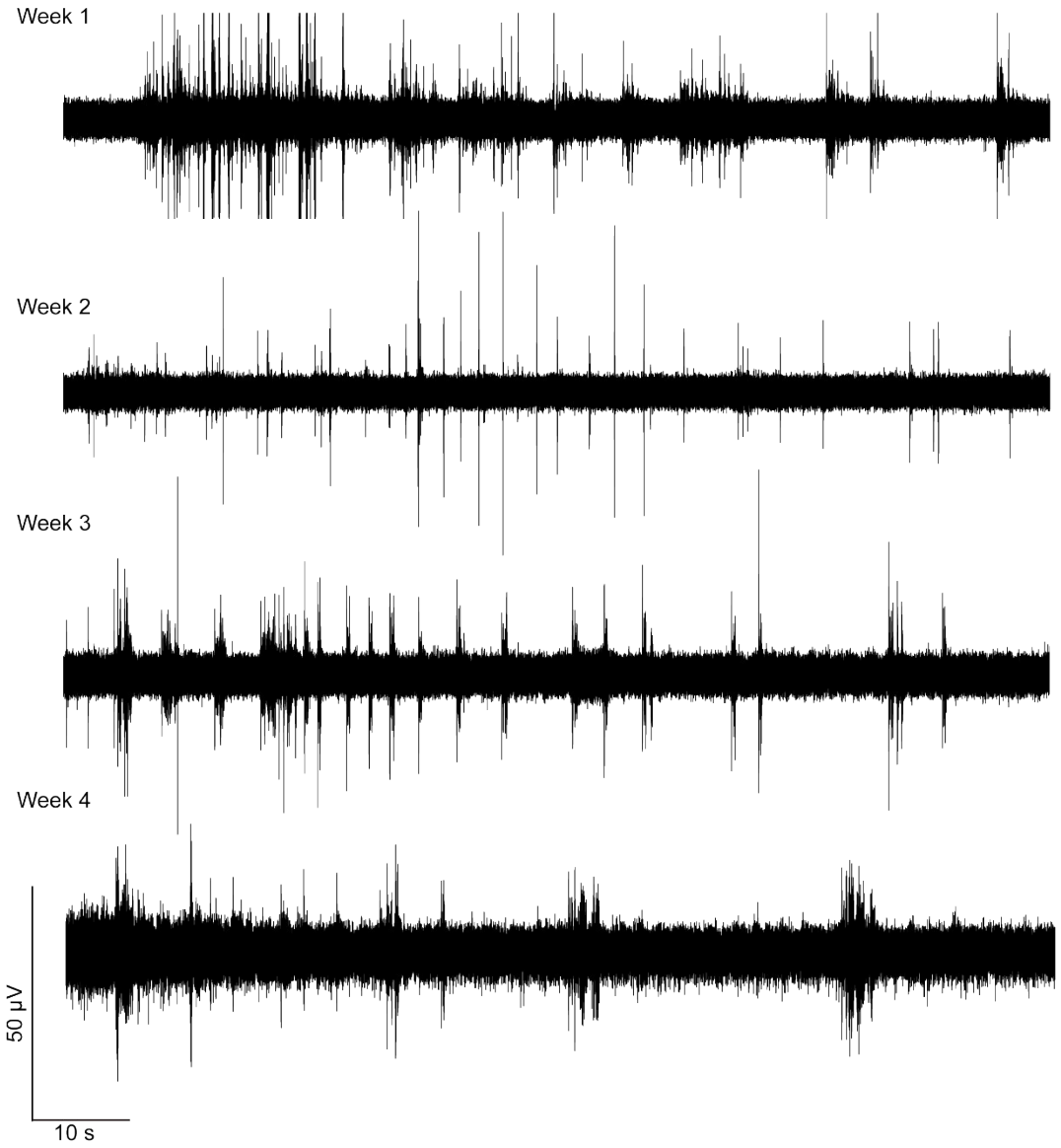

Figure S19. Neural signal recording of a MFNP *in vivo* for 4 weeks.

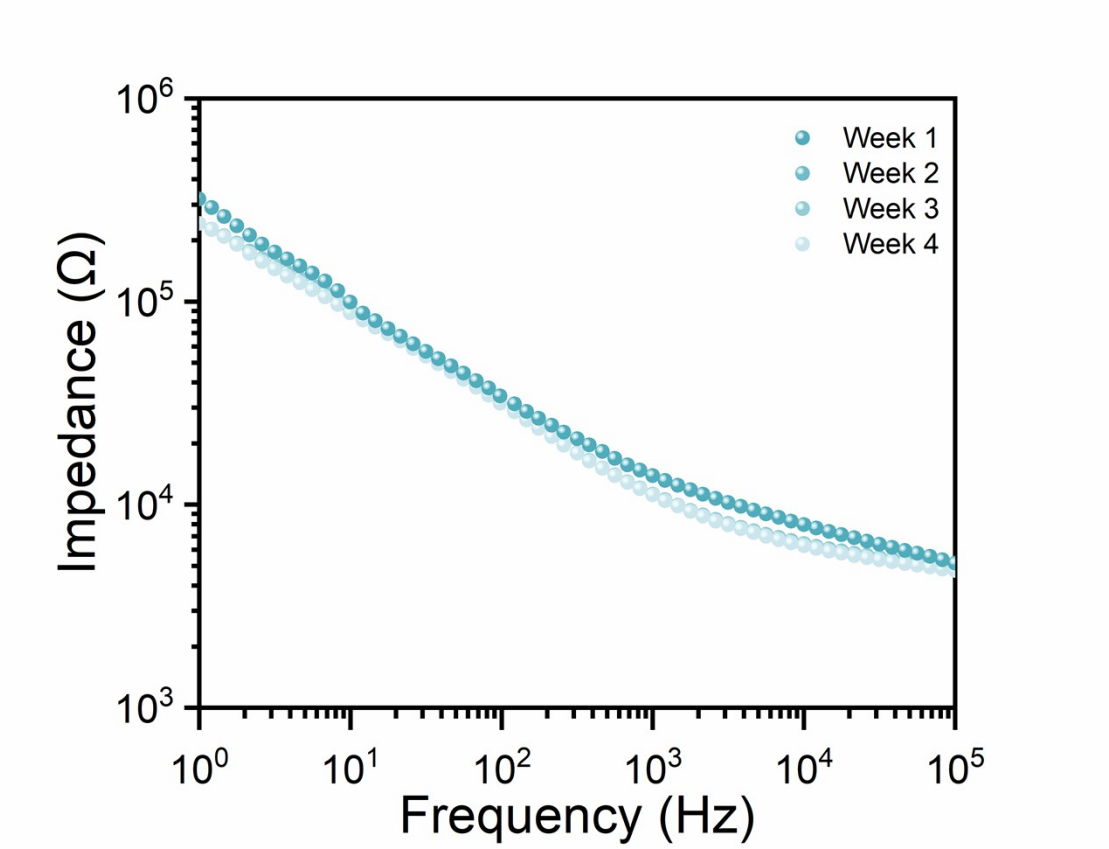

Figure S20. Impedance spectra of a Wet-MFNP immersed in artificial cerebrospinal fluid of mice for 4 weeks.

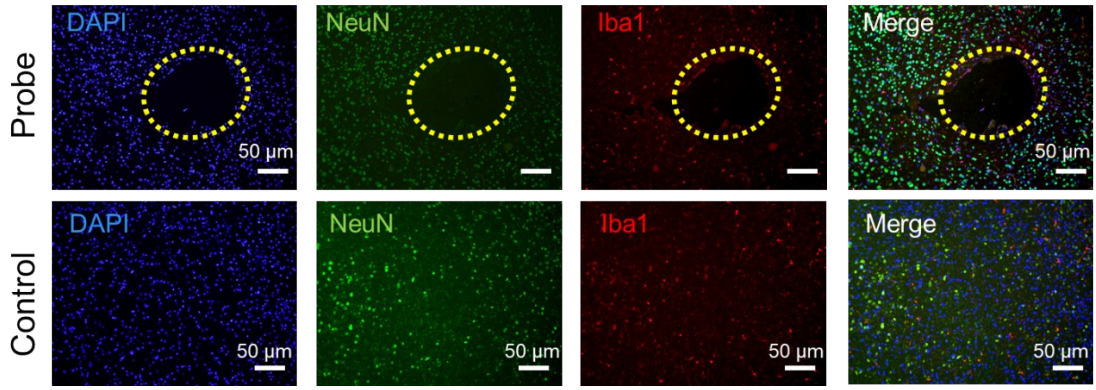

Figure S21. Immunohistochemical staining of a horizontal brain slice after 7-day implantation of the MFNP and control group without implants. The 100-μm-thick slice was labeled for DNA (DAPI, blue), neurons (NeuN, green), activated macrophages (ionized calcium-binding adaptor molecule 1 (Iba1), red), and overall lesion. The yellow dotted cycle indicates the position of MFNP.

Table S1. The elastic modulus and specific impedance of state-of-art neural electrodes.

Materials	Modulus (GPa)	Specific Impedance (MΩ μm^2)	Reference
W	~200	25	(S1) (S2)
Pt	105.75	424	(S3) (S4)
PtIr	103.92	452	(S4) (S5)
IrO_x	-	23.54	(S6)
NiCr	194	~600	(S5) (S7)
TiN	~640	155	(S8) (S9)

Reference

S1 J. C. Williams, J. A. Hippensteel, J. Dilgen, W. Shain and D. R. Kipke, *J. Neural Eng.*, 2007, **4**, 410–423.

S2 S. P. Lacour, G. Courtine and J. Guck, *Nat. Rev. Mater.*, 2016, **1**, 16063.

S3 Y. Lu, T. Li, X. Zhao, M. Li, Y. Cao, H. Yang and Y. Y. Duan, *Biomaterials*, 2010, **31**, 5169–5181.

S4 A. Petrossians, J. J. Whalen III, J. D. Weiland and F. Mansfeld, *J. Electrochem. Soc.*, 2011, **158**, D269.

S5 F. Vitale, S. R. Summerson, B. Aazhang, C. Kemere and M. Pasquali, *ACS Nano*, 2015, **9**, 4465–4474.

S6 S. J. Wilks, S. M. Richardson-burns, J. L. Hendricks, D. C. Martin, K. J. Otto, *Front. Neuroeng.* 2009, **2**, 1.

S7 X. Jin, L. Wu, G. Licheng and S. Yuguo, *Compos. Sci. Technol.*, 2009, **69**, 1587–1591.

S8 J. D. Weiland, D. J. Anderson and M. S. Humayun, *IEEE Trans. Biomed. Eng.*, 2002, **49**, 1574–1579.

S9 E. Török, A. J. Perry, L. Chollet and W. D. Sproul, *Thin Solid Films*, 1987, **153**, 37–43.