Electronic Supplementary Material (ESI) for Materials Horizons.

Supplementary Information

Flexible Silk Fibroin-Based Microelectrode Arrays for High-Resolution Neural Recording

Jie Ding, [a] Mingze Zeng, [a], Yuan Tian, [a] Zhihong Chen, [a] Zi Qiao, [a] Zhanwen Xiao, [a] Chengheng Wu, [a,] [b] Dan Wei, * [a] Jing Sun, [a] Hongsong Fan* [a]

[a] *National Engineering Research Center for Biomaterials, College of Biomedical Engineering, Sichuan University, Chengdu 610064, Sichuan, P. R. China*

[b] *Institute of Regulatory Science for Medical Devices, Sichuan University, Chengdu 610064, Sichuan, P. R. China*

* Corresponding author email: Dan Wei (*danwei@scu.edu.cn*) and Hongsong Fan (*hsfan@scu.edu.cn*)

Experimental section

Materials

Bombyx mori was obtained from Zhejiang Academy of Agricultural Sciences (China). Polystyrene sulfonate (PEDOT: PSS, PH 1000, 1.1% in H_2O) was purchased from Clevios (Germany). Dimethyl sulfoxide (DMSO, AR, ＞99%) and hydrofluoric acid (HF, AR, ＞ 99%) were purchased from Chengdu Kelong Chemical Research Factory (China). Al_3TiC_2 (≥90%) and poly(ethylene glycol) diglycidyl ether (PEGDE, Mn 520~550) were got from Aladdin Scientific Corp. (China).

Fabrication of crosslinked SF substrate

The extraction of SF was conducted according to our previous report.[28] Afterwards, preparation of crosslinked SF film by casting method. 200 mg of freeze-dried SF was dissolved in 5 mL of deionized water to prepare a 4 wt% SF aqueous solution, which was then added to a 50 mL single-necked flask. Subsequently, 20 wt%, 30 wt%, and 40 wt% PEGDE were added, and the mixture was reacted at 60°C for 1 hour. Finally, the reaction solution was added to a square weighing boat with sides of 2.5×2.5 cm, and air-dried to obtain the crosslinked SF film (CSF).

Fabrication of Ti_3C_2 nanosheets

3g of Ti_3AlC_2 was gradually added to an etching solution (250 mL of 12M HCl, 4.8 g of LiF to 60 mL) under ice bath, followed by continuous stirring at room temperature for 24 hours. The centrifuged product was washed to near pH=6, then the solution was manually shaken until a large amount of bubbles were produced, the delaminated Ti_3C_2 aqueous dispersions were obtained. Finally, the Ti_3C_2 powder was obtained by vacuum drying at 60°C for 24 hours, which was then used for the preparation of screen-printing ink.

Screen printing of SF-based microelectrode array

Ti_3C_2 nanosheets were mixed with a small amount of conductive carbon paste to give them curing and shaping properties. CSF film of about 100 μm thickness was fixed on the printing platform. By adjusting the force and angle of the scraper, the Ti_3C_2 and

carbon paste conductive ink were uniformly printed on the CSF. Subsequently, the PEDOT: PSS solution was printed on the electrode points and further immersed into DMOS solution, finally another CSF layer of insulation film was encapsulated to obtain the SMEA.

Characterization

The morphology of the prepared Ti_3C_2 nanosheets was observed using a transmission electron microscope (JEM-1400 PLUS, JEOL Ltd., Japan). Dynamic mechanical analysis (DMA) was conducted on crosslinked SF with an amplitude of 20 mm and a preload of 5 mN using a TA-Q800 instrument (US). A scanning electron microscope (SEM, S-4800, Hitachi, Japan) was used with an accelerating voltage of 5 kV to observe the morphology of screen printed Ti_3C_2 conductive phase. Dynamic light scattering (DLS) was used to characterize the particle size of the synthesized titanium carbide nanosheets. A solution of the material with a concentration of 0.1 mg/mL in deionized water was prepared and placed in a cuvette. The particle size characterization was performed using DLS (Malvern Nano-ZS90). The morphologies of the PEDOT: PSS coated Ti_3C_2 were investigated by atomic force microscopy (AFM) utilizing contacting mode with a 5 μm scanner. The crystal structure of Ti_3C_2 was characterized by XRD (Philips X'Pert Pro MPD) with Co Kα = 1.7890 Å. The prepared titanium carbide powder was ground in a mortar and pestle, and 10 mg of the sample was pressed into a pellet for testing. X-ray photoelectron spectroscopy (XPS): A certain amount of Ti_3C_2 water solution (1 mg/mL, 50 mL) was freeze-dried to obtain dried Ti_3C_2 powder, which was then characterized for the valence states and elemental composition using X-ray photoelectron spectroscopy (ESCALAB 250X 100, Thermo Fisher, USA).

Electrical and electrochemical properties

The Gamry Reference 600 potentiostat (Gamry Instruments, USA) was used for electrochemical impedance spectroscopy (EIS) and current injection capability (CIC) measurements of SMEA, which was connected in a standard three-electrode configuration in 0.1 M PBS solution. For EIS measurements, each channel in SMEA served as the working electrode, with Ag/AgCl as the reference electrode, and Pt as

the counter electrode, scanning frequencies from 10 Hz to 100 kHz. Similarly, CIC of SMEA was measured by loading ten consecutive 0.5 V biphasic voltage pulses, and the transient currents generated were recorded.

Biocompatibility

Bone marrow stem cells (BMSCs) were extracted from the bone marrow of SD rats using α-MEM high-glucose medium (Hyclone, USA) supplemented with fetal bovine serum (10%, Hyclone, USA) and penicillin/streptomycin (1%, Hyclone, USA) in a thermostatic cell incubator (5% CO_2, USA). 37°C) were cultured in medium and passed every three days.

For MTT measurement, after control group, CSF, CSF-Ti_3C_2 and SMEA were co-cultured with BMSCs, respectively, the extracts were incubated with MTT (10%) at 37°C for 4 hours, and then dimethyl sulfoxide (DMSO) was added and incubated for 15 min. The absorbance at 490 nm was measured with a multi-detection enzyme marker (Bio-Rad 550). For cell proliferation tests, the extracts of control group, CSF, CSF-Ti_3C_2 and SMEA were co-cultured with BMSCs for 1, 3 and 5 days, and the morphology of BMSCs was detected by FDA/PI staining. The cell survival was observed by inverted fluorescence microscope (Leica, DMi8 A, German). For the anti-protein absorption, control group, CSF, CSF-Ti_3C_2 and SMEA were immersed in the prepared BSA solution (0.5 mg mL^{-1}) and concentrations of BSA were obtained by measuring absorbance at 562 nm with a BioTek Instruments Inc. (USA).

Animal experiments

All the experiments were approved by the Medical Ethics Committee of Sichuan University (KS2022863). Sciatic nerve stimulation and recording: after anesthetizing SD rats with 10% chloral hydrate, shaving the hair, and dissecting the biceps femoris and semitendinosus muscles to expose the sciatic nerve, the SMEA was placed on the exposed sciatic nerve for sciatic nerve evoked potential recording. Mechanical stimulation including poking, pinching, and scratching the rat's ankle joint was applied for about 2 seconds each, and using the SMEA for recording. The other end of the SMEA was connected to a 128-channel neural acquisition processor (Blackrock, USA)

amplifier and host computer for transmitting neural field potential signals. The sampling rate of LFP was 1 kS/s, and the band-pass filtering frequency was 1-250 Hz.

Epilepsy modeling and electrophysiological recording: using 4-AP as a drug to induce epilepsy, SD rats were peritoneally anesthetized with 10% chloral hydration (350 mg/kg). After disinfection, shaving, craniectomy, and dural incision, a 4 mm^2 area was exposed on the surface of the cerebral cortex, located at the projection site of the left hippocampal CA3 (AP, 5.5 mm; ML, 4.5 mm, DV, 5 mm). Then, placing the SMEA onto cortex to record the LFP at different seizure stages. The sampling rate of LFP was 1 kS/s, and the band-pass filtering frequency was 1-250 Hz. NeuroExplorer software was used to process and analyze the original neural signals.

Flash visual-evoked potential recording: SMEA was placed in the visual cortex V1 area of rats, and white light stimulation at a frequency of 1 Hz was applied to the rat's eyes to induce discharge of visual cortex neurons. The sampling rate was 30 kS/s, and the bandpass filter width was BP 250 kHz. The BOSS software was used for sorting neurons, and the K-means algorithm was used for clustering analysis of neuronal signals. Signal analysis and processing were completed using NeuroExplorer software.

Statistical analysis

Statistically analysis: Statistical analysis was performed with Origin software and Graph Pad 7.0. Statistical significance was measured by one or two-way ANOVA test and significance level were set at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), $p<0.0001$.

Additional Results

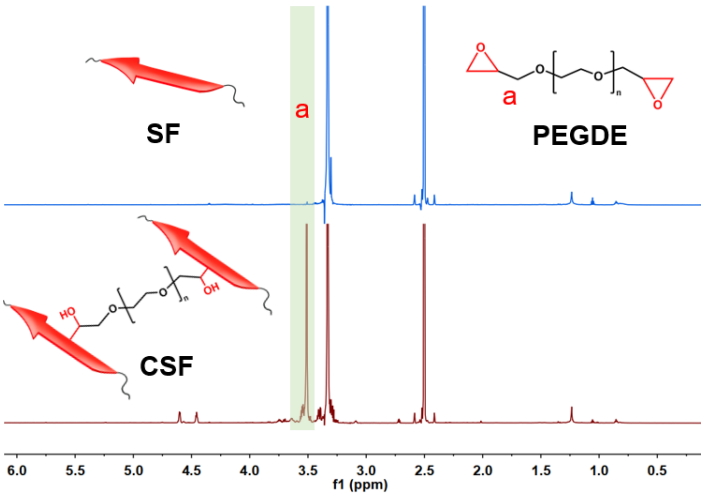

Figure S1 ^{1}H-NMR spectrum of PEGDE crosslinked SF.

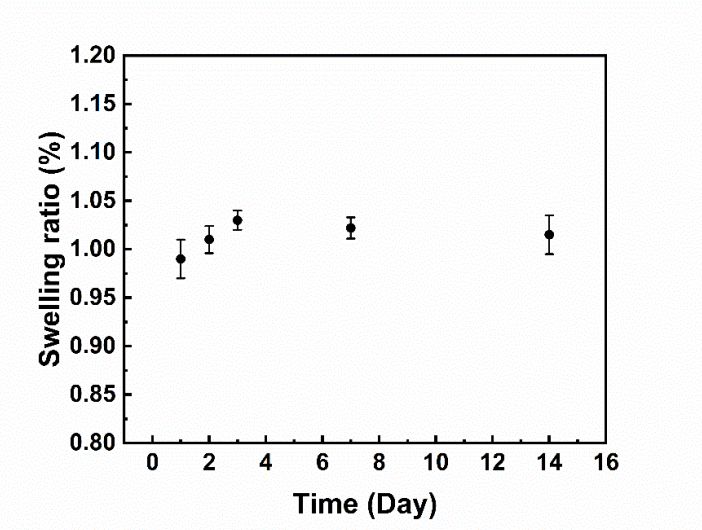

Figure S2 The swelling ratio of PEGDE (30 wt%) crosslinked SF.

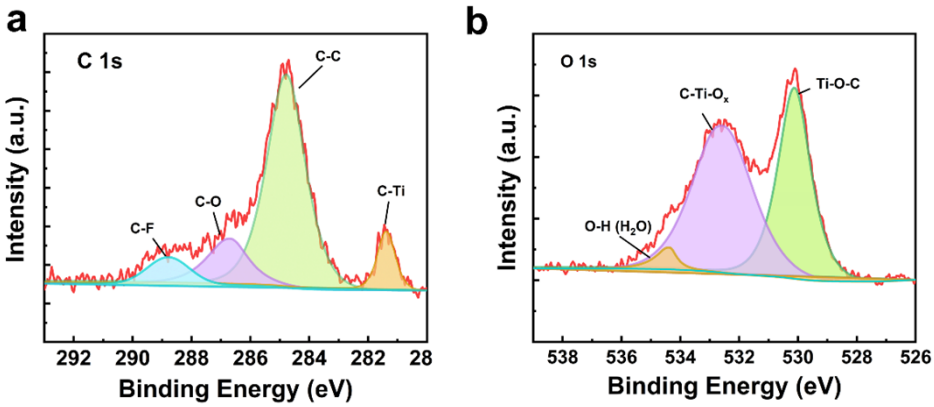

Figure S3 XPS spectrums of prepared Ti_3C_2. (a) C1s and (b) O1s of Ti_3C_2.

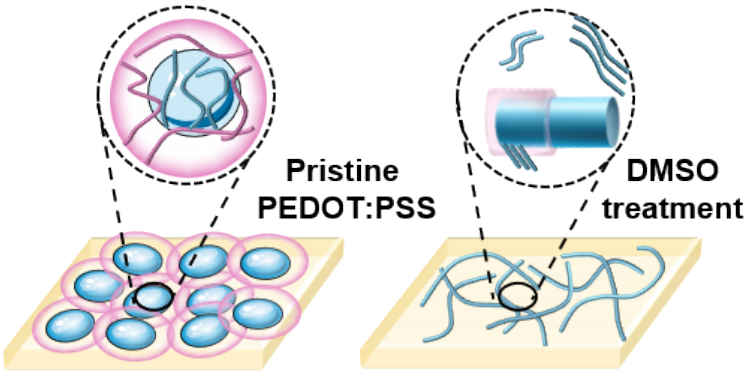

Figure S4 Schematic illustration of DMSO treatment of PEDOT: PSS coated Ti3C2 electrode points

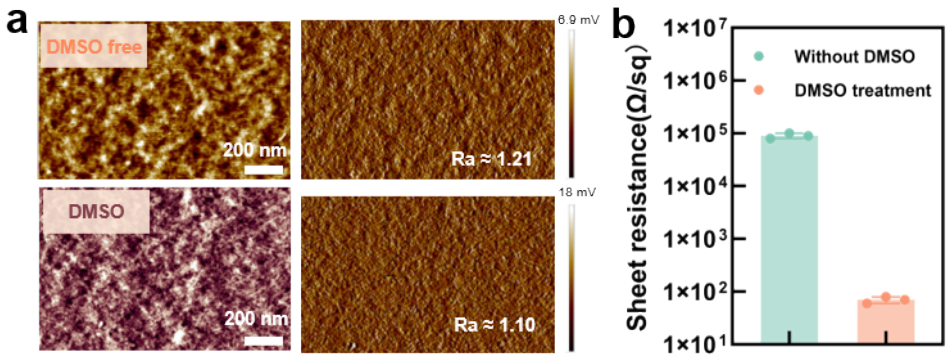

Figure S5 (a) AFM images and (b) Sheet-resistance of PEDOT: PSS before and after DMSO treatment.

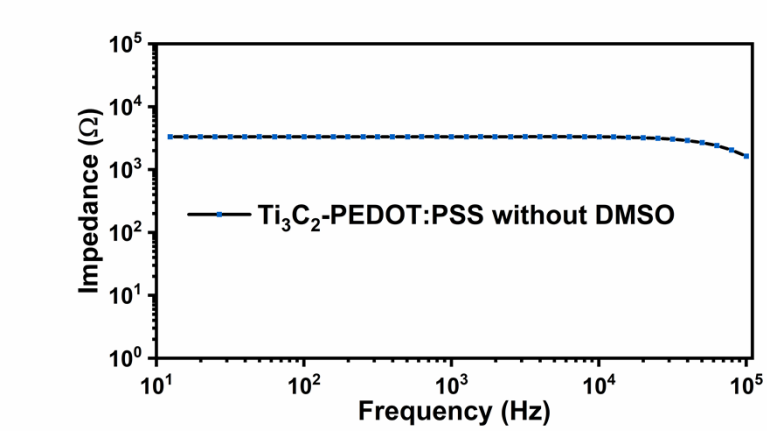

Figure S6 The impedance curve of Ti_3C_2-PEDOT:PSS without DSMO treatment.

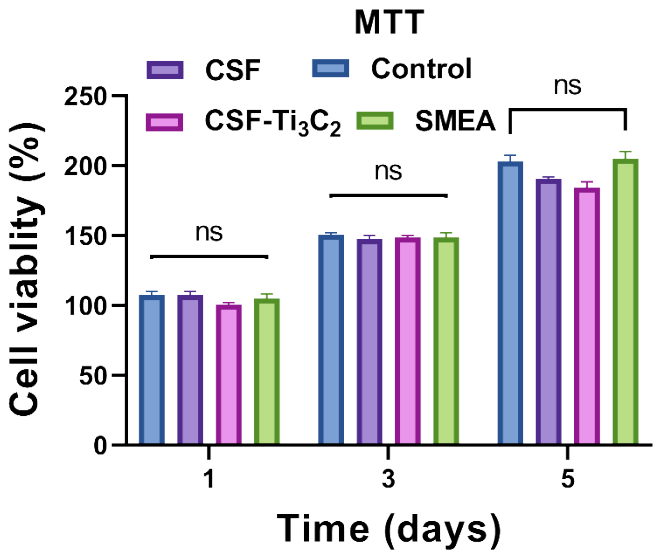

Figure S7 MTT test for proliferation of BMSCs in three groups of SF-based materials (n=3)

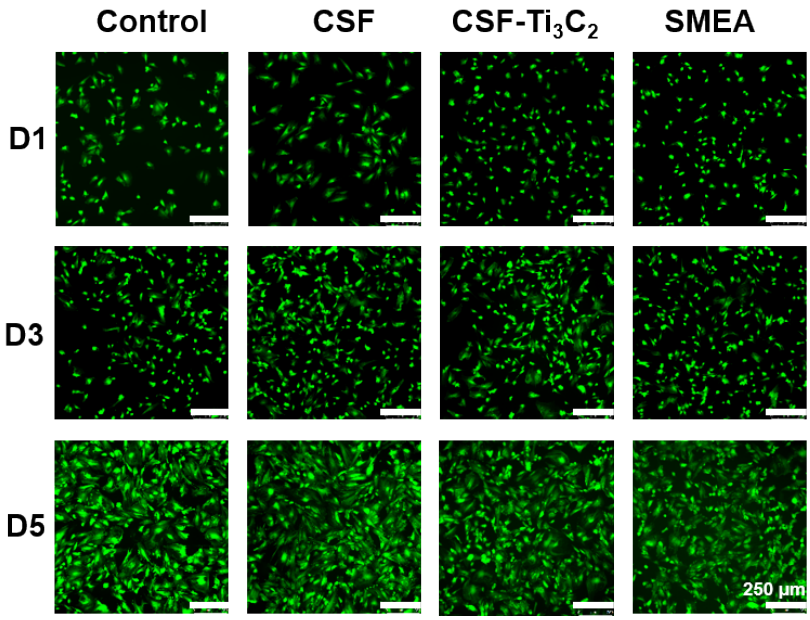

Figure S8 FDA (green)/PI (red) staining of BMSCs co-cultured in three groups of SF-based materials after 1, 3, and 5 days.

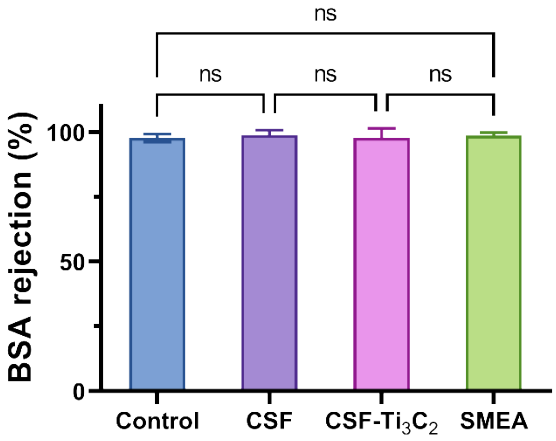

Figure S9 BSA rejection three groups of SF-based materials.

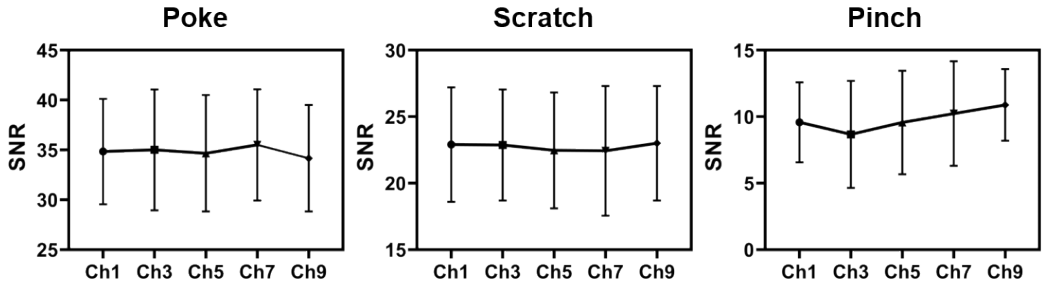

Figure S10 The SNR values of muscle electromyographic signals recorded by SMEA in response to different mechanical stimuli.

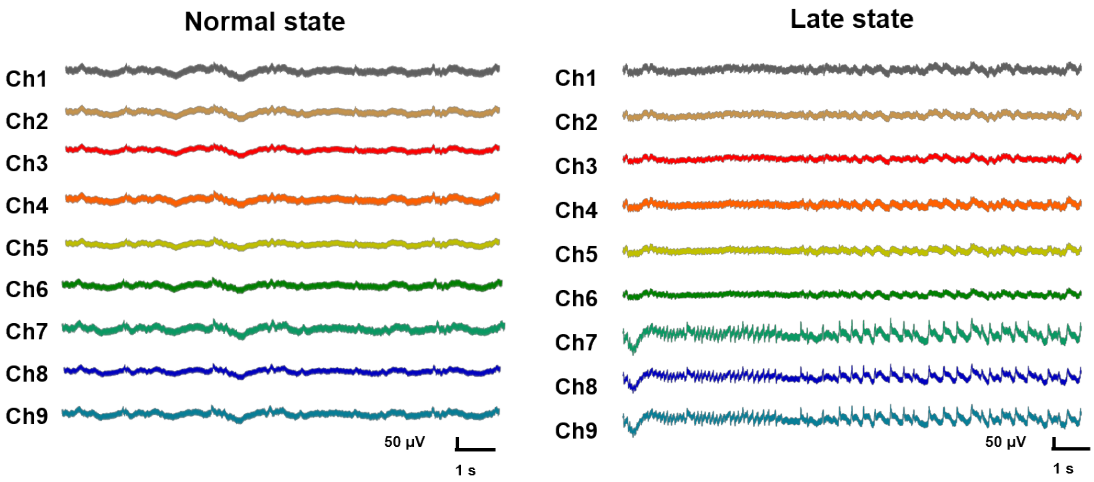

Figure S11 Electrocorticography signals of rats in the normal and late stage of acute state epilepticus recorded by SEMA.

Table 1 Young's modulus of commercial available polymer films

Polymer films	Young's Modulus
Parylene C	2.8 - 4.5 GPa[1]
PI	2.4 - 3.2 GPa[2]
PLGA	203 MPa[3]
CSF (this work)	1 MPa

Reference

(1) Galeotti F.; Andicsova A., Bertini F., et al. Enhanced Elasticity in Parylene Thin Films by Copolymerization Approach. *J. Mater. Sci*., **2014**, *49* (21): 7547-7555.

(2) Singh B. P.; Singh D.; Mathur R. B., et al. Influence of Surface Modified MWCNTs on the Mechanical, Electrical and Thermal Properties of Polyimide Nanocomposites, *Nanoscale Res. Lett*., **2008**, *3* (11): 444-453.

(3) Park J. J.; Yu E. J.; Lee W. K., et al. Mechanical Properties and Degradation Studies of Poly(D, L-lactide-co-glycolide) 50:50/Graphene Oxide Nanocomposite Films, *Polym. Adv. Tech*., **2014**, *25* (1): 48-54.