Electronic Supplementary Material (ESI) for Biomaterials Science.

Hierarchically patterned protein scaffolds with nano-fibrillar and micro-lamellar structures modulate neural stem cell homing and promote neuronal differentiation

Jiaqi Hu [a], Chenlin Li [a], Zhangze Yang [a], Qi Wu [a], Jie Wang [a, b], Zongpu Xu [a, b], Yuyin Chen [a, b], Quan Wan [a, b], Yajun Shuai*[, a, b], Shuxu Yang*[, c], Mingying Yang*[, a, b]

a Institute of Applied Bioresource Research, College of Animal Science, Zhejiang University, Hangzhou, Zhejiang 310058, China

b Key Laboratory of Silkworm and Bee Resource Utilization and Innovation of Zhejiang Province, Hangzhou, Zhejiang, 310058, China

c Department of Neurosurgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, Hangzhou, 310016, China

* Corresponding author.

Supplementary materials

Experimental methods

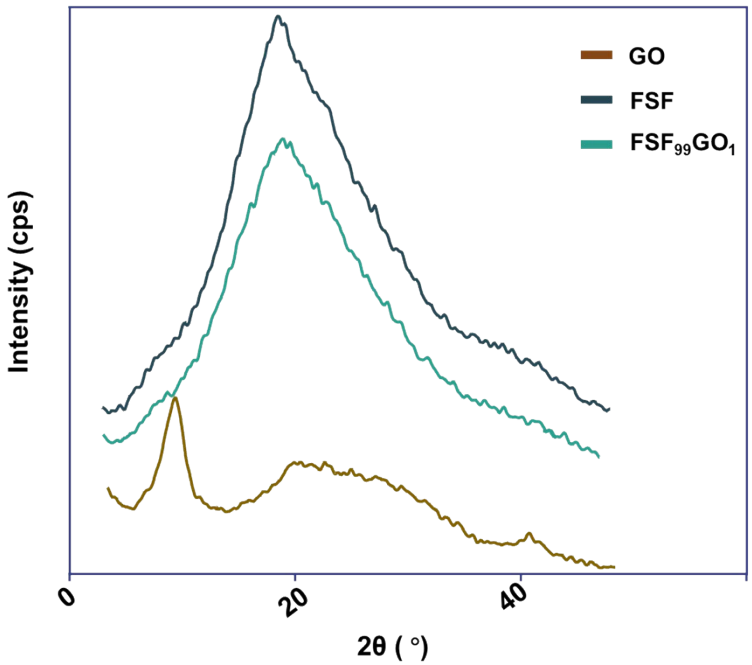
Supplementary Figures 1-12

Experimental methods

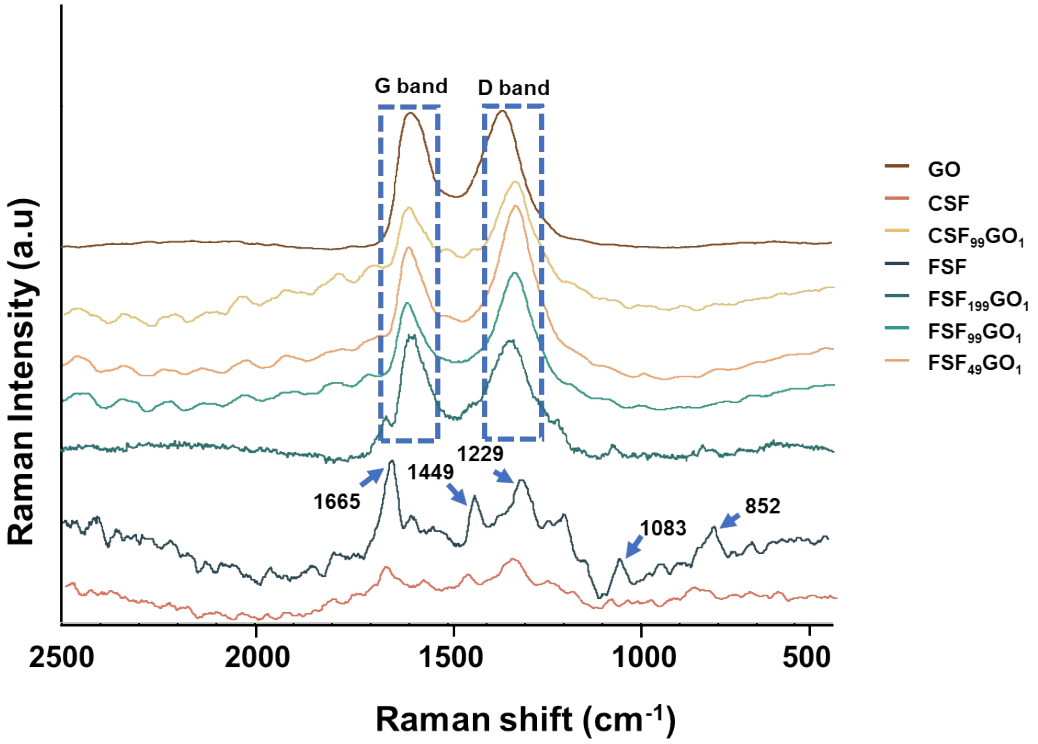
Preparation of SF-GO film: SF film was prepared by extension method with 2% solution. After ultrasonic dispersion for 30 min, 2% GO solution was well-dispersed. Then SF film was immersed into the GO solution, slowly oscillating at room temperature to form the nano structure on the film surface. After processing for two hours, the SF-GO film was washed twice and air dried.

The viability of NSCs on the SF-GO film: After co-cultured with the material for 24 h, the LDH cytotoxicity of NSCs was detected by LDH Cytotoxicity Assay Kit (Beyotime, China) according the manufacturer's instructions. The cell cultured in serum-free medium on the plate without any treatment was treated as the control group. To examine the viability of NSCs on the SF-GO film, the Live/Dead assay was conducted using 1 μmol/L calcein AM and 4 μmol/L ethidium homodimer-1 (Life Technologies kit) for 30 min at 37 °C.

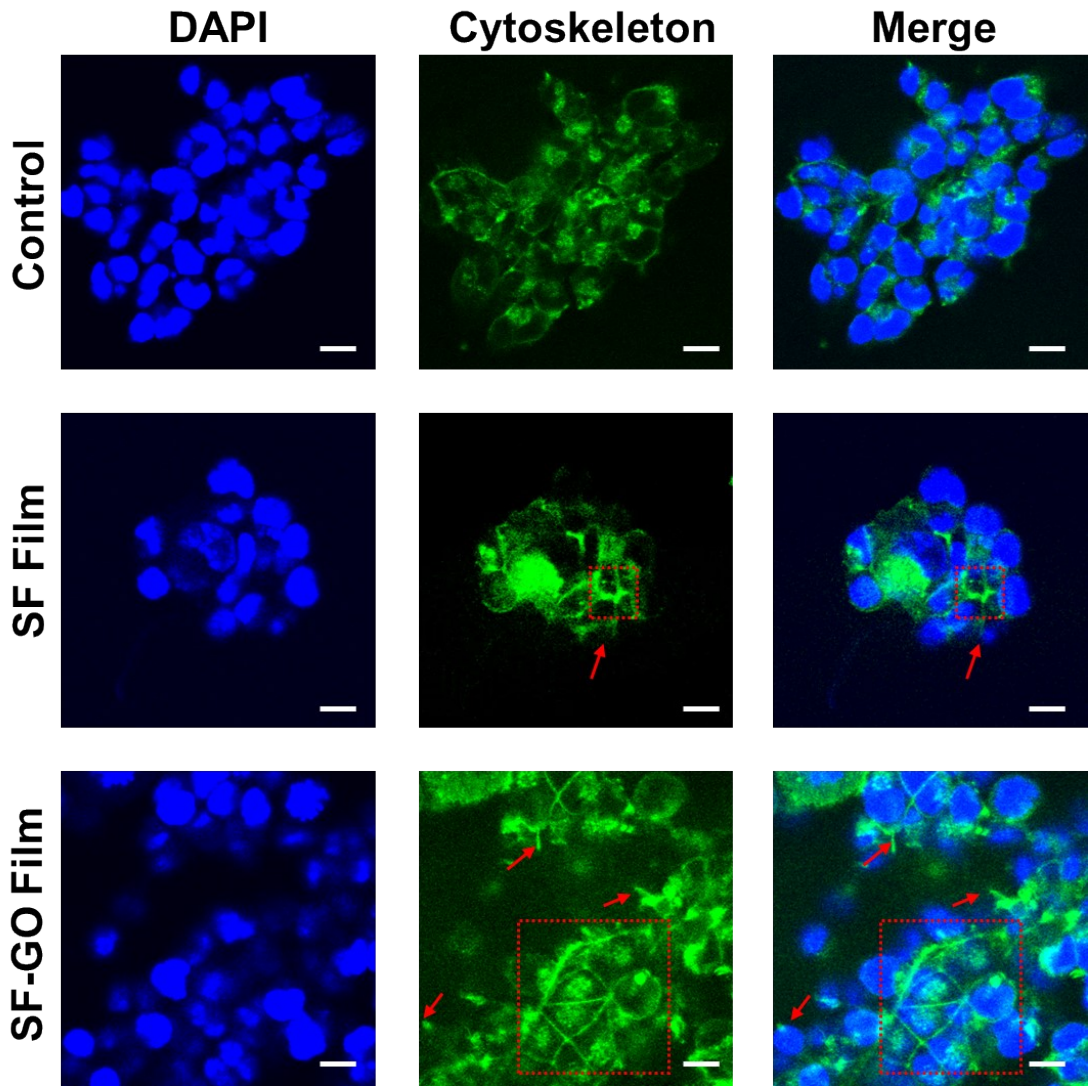
RNA extraction, Library preparation, and Sequencing: Total RNA was extracted from the NSCs growing on the scaffolds using TRIzol® Reagent according the manufacturer's instructions. Then RNA quality was determined by 5300 Bioanalyser (Agilent) and quantified using the ND-2000 (NanoDrop Technologies). Only high-quality RNA sample (OD260/280=1.8~2.2, OD260/230≥2.0, RIN≥6.5, 28S:18S≥1.0, >1μg) was used to construct sequencing library. mRNA libraries were generated by Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus. They were then sequenced using an Illumina NovaSeq 6000.



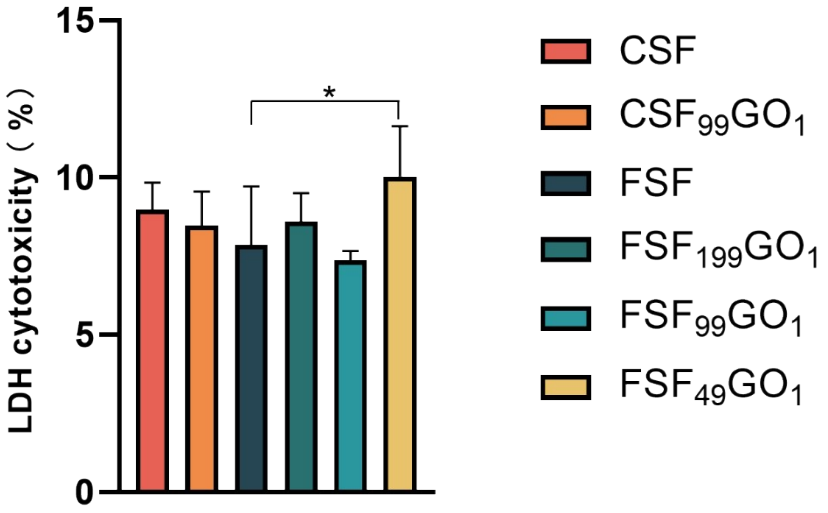
Supplementary Figure 1. The XRD patterns of GO, FSF and $FSF_{99}GO_1$ scaffolds. The peak value of GO, FSF and $FSF_{99}GO_1$ appeared at 11°, 21.4° and 20.6°, respectively.



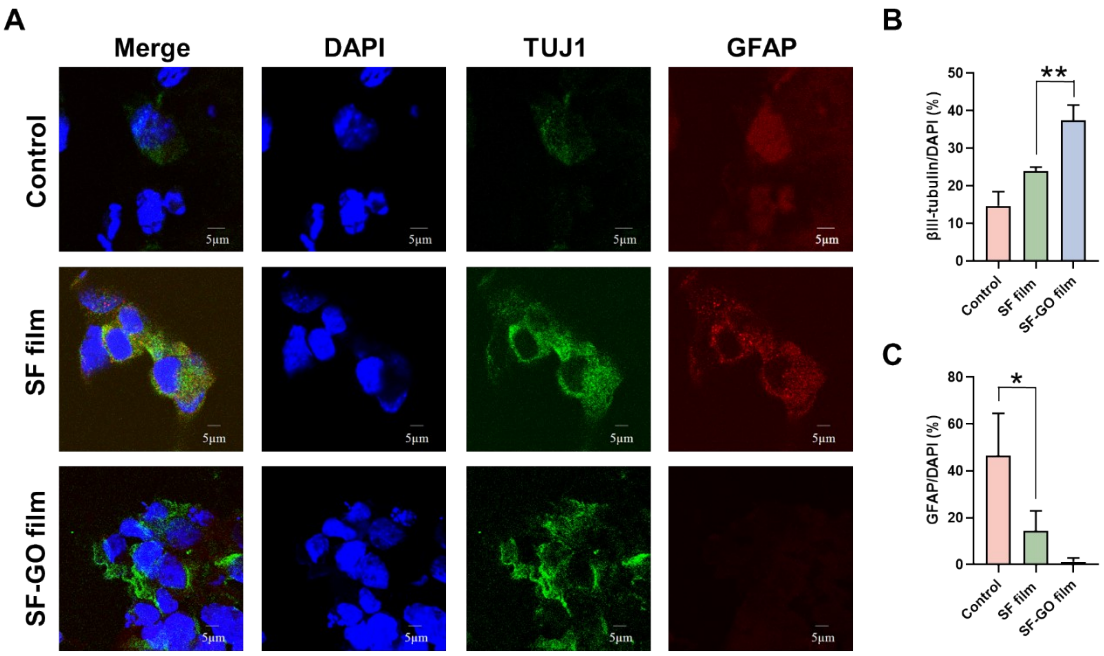
Supplementary Figure 2. The Raman spectra of GO, CSF, $CSF_{99}GO_1$, FSF, $FSF_{199}GO_1$, $FSF_{99}GO_1$ and $FSF_{49}GO_1$ scaffolds. FSF and CSF scaffolds below showed random coil structure. When GO was added, the G band and D band appeared.



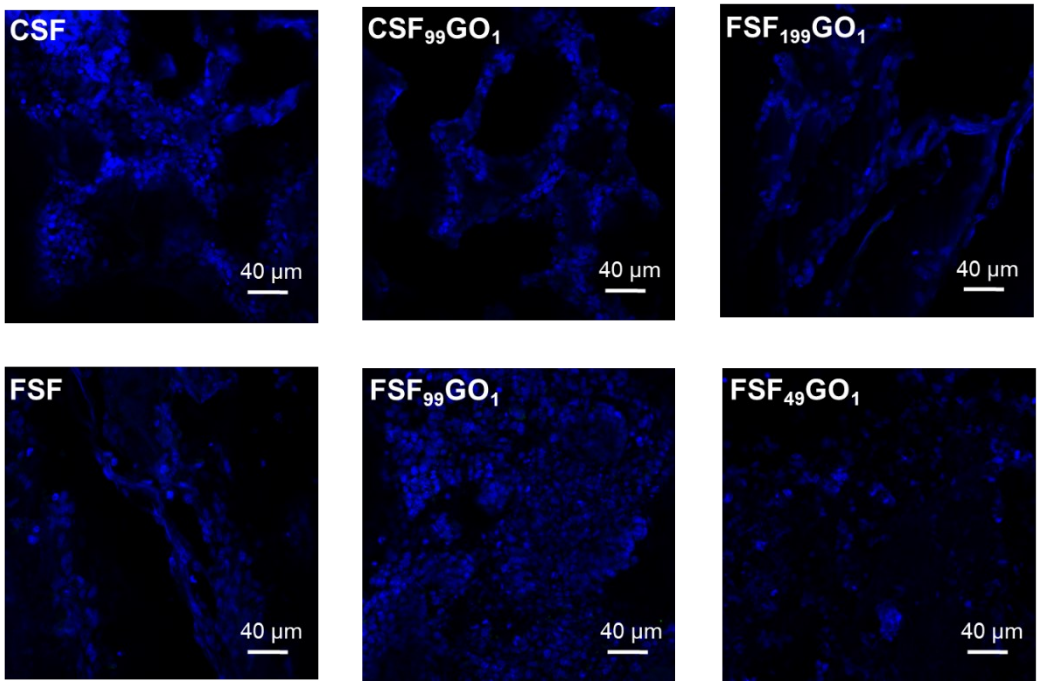
Supplementary Figure 3. Fluorescence staining images of NSCs proliferating on SF-GO film for 3 d. (Blue: nucleus; Green: cytoskeleton) Scale bars represent 20 μm. Dotted box: cells showing spindle spread. Arrow: cell pseudopodia.



Supplementary Figure 4. LDH cytotoxicity of NSCs co-cultured with the material for 24 h. Sample pairs (CSF vs $CSF_{99}GO_1$, FSF vs $FSF_{99}GO_1$, CSF vs FSF, $CSF_{99}GO_1$ vs $FSF_{99}GO_1$) were analyzed with the Student's t-test. One-way ANOVA analysis was applied among FSF-GO groups. *P<0.05, (Mean ± SD, n=3)



Supplementary Figure 5. Immunofluorescence-stained neural cells cultured on SF-GO films for 14 d. (A) Neurons are stained green (beta III tubulin: green), astrocytes are stained red (GFAP: red) and nuclei are stained blue (DAPI: blue). (B) Percentage of tubulin versus DAPI-stained area. (C) Percentage of GFAP versus DAPI-stained area. **P*<0.05, ***P*<0.01. (Mean ± SD, n=3)



Supplementary Figure 6. Immunofluorescence-stained neural cells cultured for 14 d. Astrocyte stained red (GFAP: red) and nuclei stained blue (DAPI: blue).