Supplementary materials

Baicalein-functionalized collagen scaffolds direct neuronal differentiation toward enhancing spinal cord injury repair

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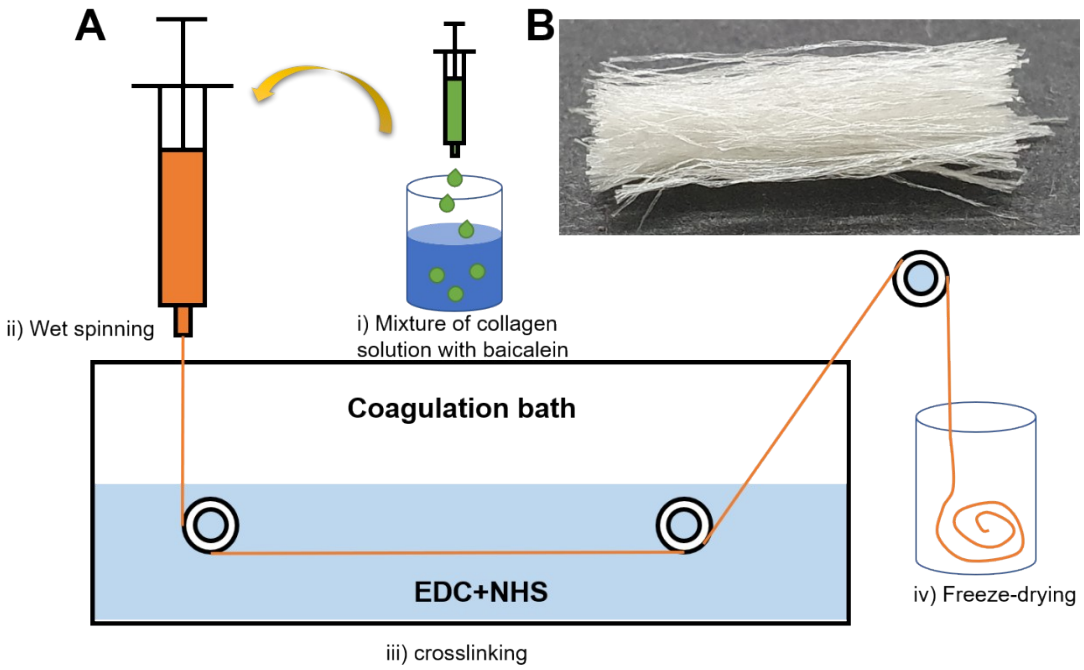
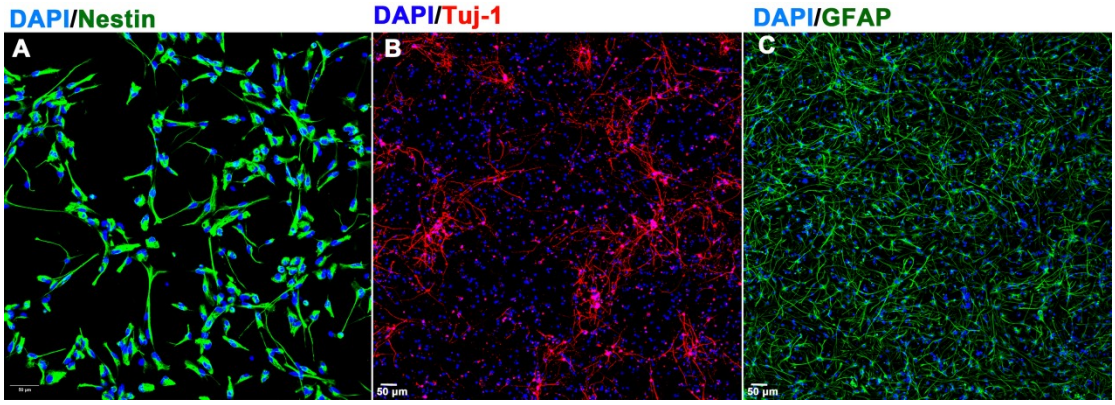
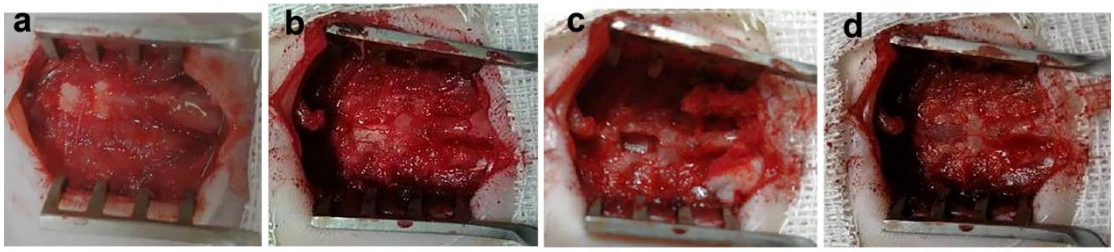


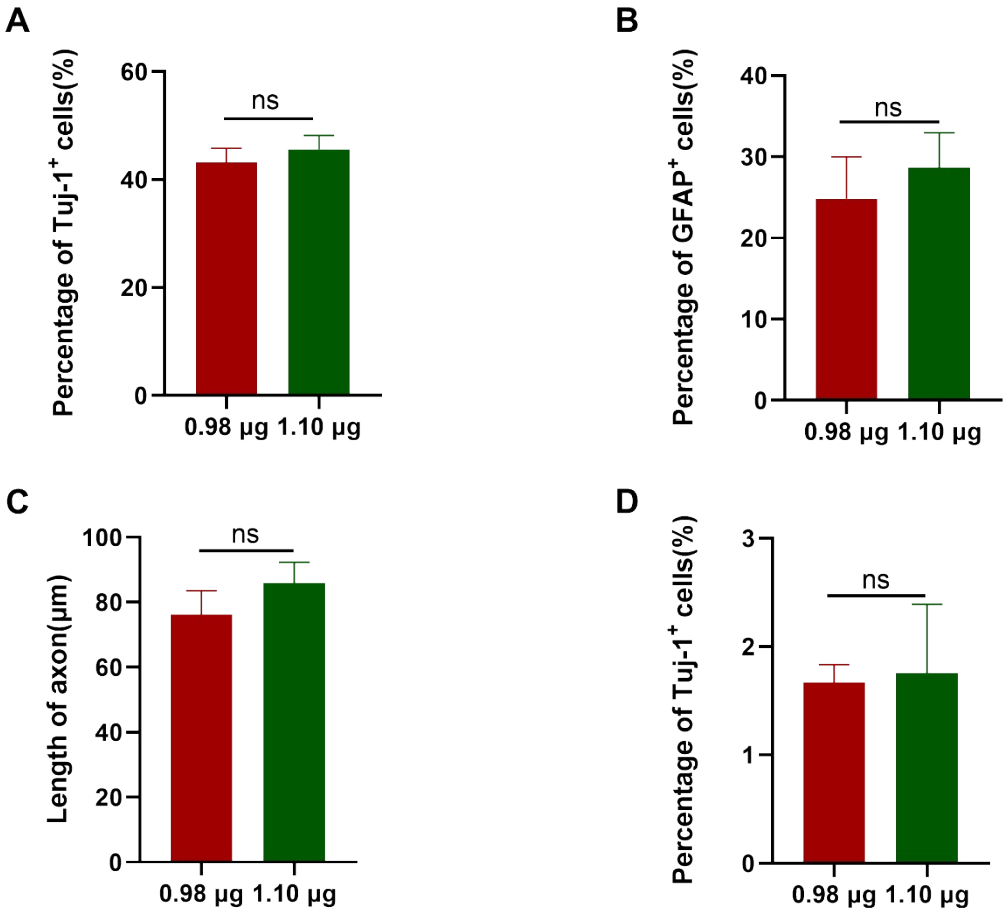
Supplementary Figure S1. (A) Schematic illustration of the BFCS preparation process. This includes 4 steps: i) the mixture of baicalein dissolved in DMSO with a collagen solution; ii) wet spinning of the mixed solution under room temperature; iii) collagen crosslinking in the coagulation bath containing EDC and NHS at 4 ℃ overnight; iv) collection of the filaments and freeze-drying. (B) An optical image of the freeze-dried BFCS containing 0.98 μg baicalein.



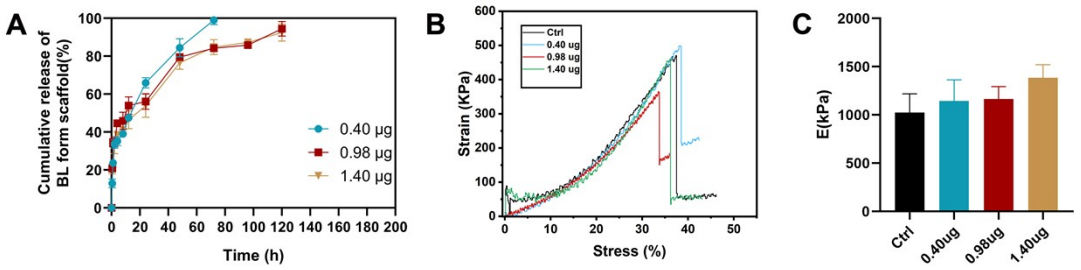
Supplementary Figure S2. (A) A representative image of neural stem cells (NSCs) at 5-day culture after dissociation from new-born rats. Cells were immunostained with Nestin. Nuclei were visualized by DAPI staining. The purity of NSCs in 3 different batches were all > 90%. Scale bar: 50 µm. (B-C) Representative images of cells differentiated from NSCs after a 7-day culture. Cells were stained with Tuj-1 to visualize newborn neurons (B) and GFAP to identify astrocytes (C). Nuclei were visualized by DAPI staining. Scale bar: 50 µm.



Supplementary Figure S3. Overview of the surgical procedures for establishing complete spinal cord transection model in rats. Pictures from left to right were the procedure of vertebrae exposure at T7-T9 segment (a-b), complete transection of 4 mm spinal cord tissue at T8 segment (c), and implantation of the BFCSs (d).



Supplementary Figure S4. Comparisons of the differentiation effects between the BFCSs containing 0.98 μg and 1.10 μg baicalein in the *in vitro* and the short-term animal experiments. In order to clearly show the effects of the two BFCSs on promoting neuronal differentiation, the proportions of Tuj-1 positive cells (A), GFAP positive cells (B) and axon length (C) seeded on the two BFCSs after a 7-day differentiation in vitro and percentage of Tuj-1 positively stained cells in the lesion area after a 7-day treatment of the two BFCSs in SCI rats (D) were separately compared. The results showed no significant difference between the two groups. The data were reproduced from Figure 3B-D and Figure 4B and the statistical difference in each panel was analyzed by Student's *t*-test.



Supplementary Figure S5. Characterization of the BFCSs (4 mg and 4 mm) loaded with 0.40 μg, 0.98 μg and 1.40 μg baicalein. (A-C) Drug release kinetics (A), representative stress-strain curves (B) and Young's modulus (C) of the BFCSs loaded with 0.40 μg, 0.98 μg and 1.40 μg baicalein. Characterization data of the BFCSs loaded with 0.98 μg and 1.40 μg baicalein were reproduced from Figure 2. Data in panel C are mean and standard deviation over 3 measurements. One-way ANOVA analysis was used in panel C and indicated that the difference across groups was not statistically significant ($p < 0.05$ was considered significant).

Supplementary Video S1-4. Videos of the movements of the SCI rats at 8-week post-surgery in the groups of control (Video S1), 0.40 μg baicalein loaded BFCS (Video S2), 0.98 μg baicalein loaded BFCS (Video S3) and 1.40 μg baicalein loaded BFCS (Video S4).