Supplementary Information

Exploring natural silk protein sericin for regenerative medicine: an injectable, photoluminescent, cell-adhesive 3D hydrogel for cell and drug delivery

Zheng Wang[#], Yeshun Zhang[#], Jinxiang Zhang, Lei Huang, Jia Liu, Yongkui Li, Guozheng Zhang, Subhas C. Kundu, Lin Wang*

[#] Co-first author;
* Corresponding author

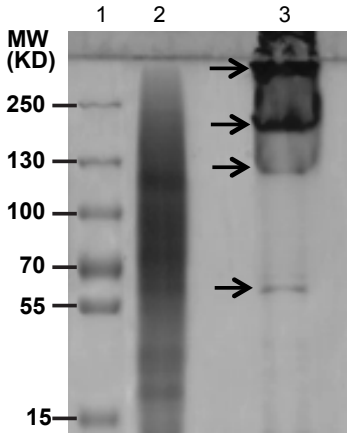

Fig. S1. The protein profiles of the sericin solution extracted using two different methods. Lane 1, protein ladders; lane 2, the sericin solution extracted from the fibroin-deficient mutant cocoons boiled for 1 hour (smear); lane 3, the sericin solution extracted from the fibroin-deficient mutant cocoons treated with 6 M LiBr at 35°C for 24 hours (four specific protein bands).

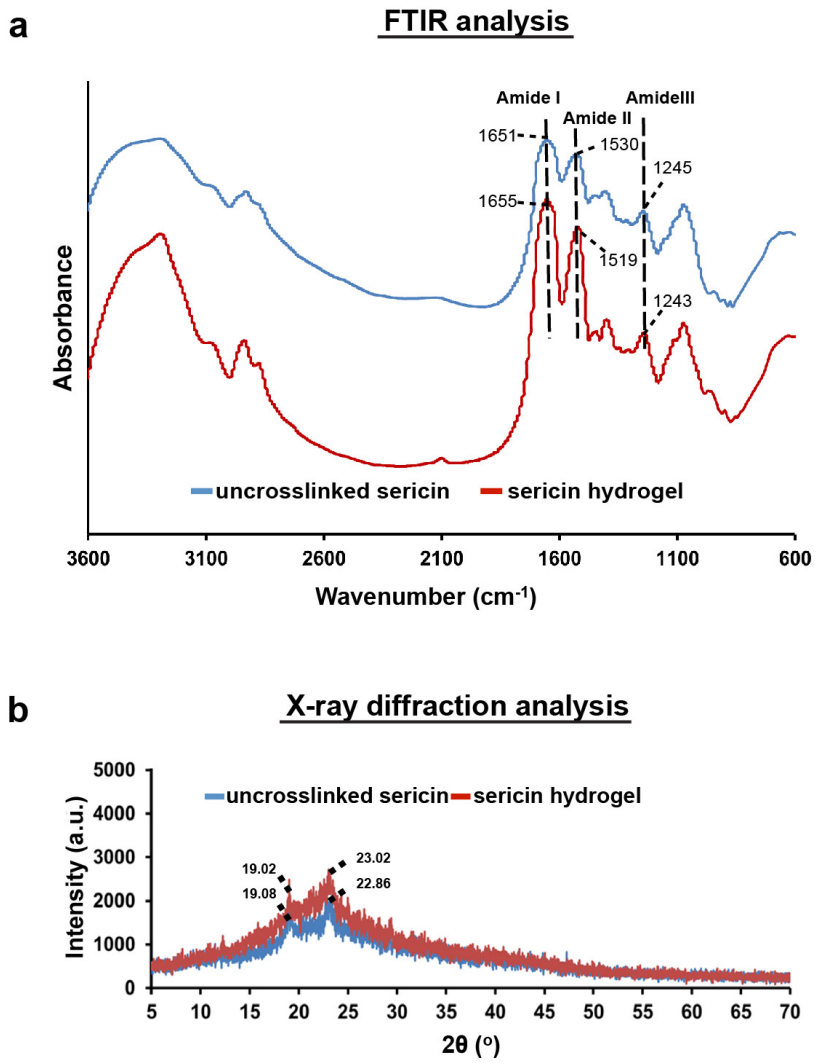

Fig. S2. FTIR and X-ray diffraction analyses on sericin protein and the crosslinked sericin hydrogel.

(a) FTIR spectra show the absorption peaks of amide I, II, and III (indicated) of uncrosslinked sericin (blue line) and a crosslinked sericin hydrogel (red line). (b) X-ray diffraction of uncrosslinked sericin (blue line) and a sericin hydrogel (red line). 2θ degrees corresponding to their peaks were indicated.

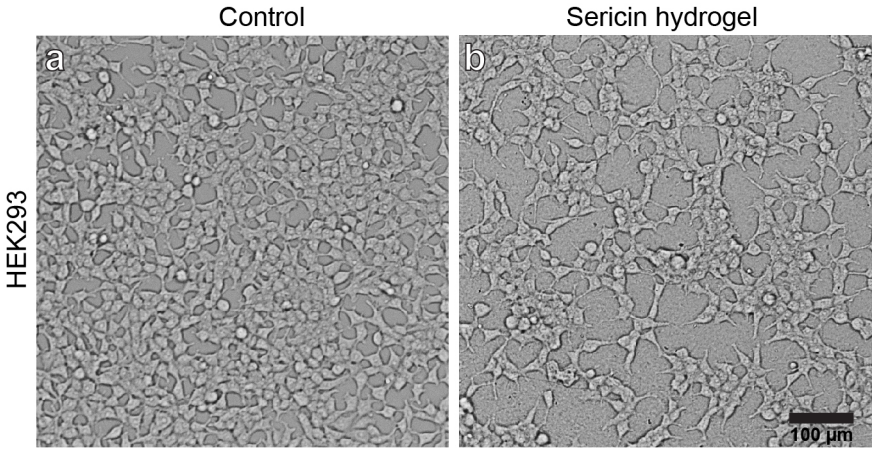

Fig. S3. Human embryo kidney cells (HEK293) grow in clusters on the surface of the sericin hydrogel. (a) HEK293 cells were seeded at the density of 3 x 10^5 on the regular 35-mm cell culture plate and grew for 24 hours. (b) HEK293 cells grew in clusters 24 hours after seeded at the density of 3 x 10^5 on the surface of the hydrogel that was formed on the surface of a regular 35-mm cell culture plate. Scale bar, 100 μm.

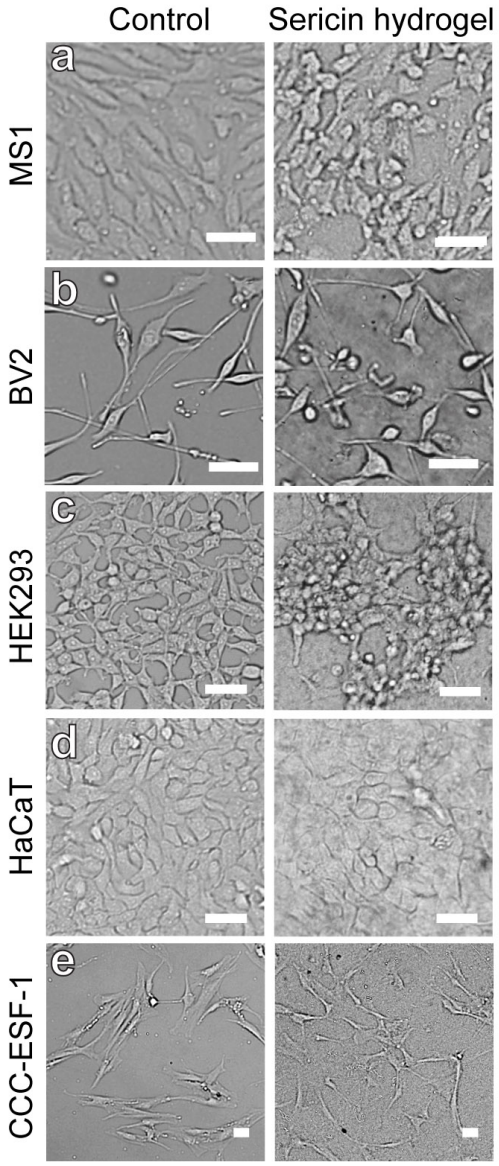

Fig. S4. The sericin hydrogel promotes the attachment and growth of various types of cells. (a-e) The morphology of five different types of cells on the polystyrene surface of the culture dishes (left column) and the sericin hydrogel (right column) at Day 4.5 after seeding. (a) Mouse pancreatic islet endothelial cells (MS1); (b) Mouse microglial cells (BV2); (c) Human embryo kidney cells (HEK293); (d) Human keratinocytes (HaCaT); (e) Human primary embryo skin fibroblasts (CCC-ESF-1). Scale bars, 50 μm.

Table S1 The related information regarding all the cell lines used in this study

Cell lines	Providers	Origin
Mouse pancreatic islet endothelial cells (MS-1) [1, 2]	Cell bank of Chinese Academy of Sciences (Shanghai, China)	Mouse normal pancreatic islet endothelial cells isolated from micro-vessels of murine endocrine pancreas and infected by SV40
Mouse myoblasts (C2C12) [3, 4]	Cell bank of Chinese Academy of Sciences (Shanghai, China)	Muscle origin
Mouse microglial cells (BV-2) [5, 6]	Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China)	Primary microglial cell (islated from brain) infected by a -*raf*/v-*myc* oncogene carrying retrovirus (J2)
Human embryo kidney cells (HEK293) [7, 8]	Cell bank of Chinese Academy of Sciences (Shanghai, China)	Human embryonic kidney cells transformed by DNA from adenovirus type 5
Human keratinocytes (HaCaT) [9, 10]	Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China)	Isolated from adult skin and infected by SV40
Human primary embryo skin fibroblasts (CCC-ESF-1) [11, 12]	Cell Center of the Institute of Basic Medical Sciences of Chinese Academy of Medical Sciences, Beijing, China	
Human umbilical vein endothelial cell / Bladder carcinoma cell line (ECV304) [a] [13-16]	China Center for Type Culture Collection (Wuhan, China)	Human umbilical vein origin or bladder carcinoma
Human umbilical vein endothelial cell (EA.hy926) [17, 18]	Cell bank of Chinese Academy of Sciences (Shanghai, China)	Human primary umbilical vein endothelial cells fused with the human lung carcinoma cell line A54

[a] This identity of this cell line is arguable. Although it is uncertain whether it derives from human umbilical vein endothelium or human bladder carcinoma, this cell line likely is epithelial origin as the umbilical vein endothelium or bladder carcinoma share epithelial origin.

Supplement References

1. LeCouter, J., et al., *Identification of an angiogenic mitogen selective for endocrine gland endothelium.* Nature, 2001. **412**(6850): p. 877-84.
2. Spelios, M.G., et al., *In vitro formation of beta cell pseudoislets using islet-derived endothelial cells.* PLoS One, 2013. **8**(8): p. e72260.
3. Yaffe, D. and O. Saxel, *Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle.* Nature, 1977. **270**(5639): p. 725-7.
4. Lathuiliere, A., et al., *A high-capacity cell macroencapsulation system supporting the long-term survival of genetically engineered allogeneic cells.* Biomaterials, 2014. **35**(2): p. 779-91.
5. Fischer, R., et al., *Astrocyte-specific activation of TNFR2 promotes oligodendrocyte maturation by secretion of leukemia inhibitory factor.* Glia, 2014. **62**(2): p. 272-83.

6. Blasi, E., et al., *Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus.* J Neuroimmunol, 1990. **27**(2-3): p. 229-37.
7. Graham, F.L., et al., *Characteristics of a human cell line transformed by DNA from human adenovirus type 5.* J Gen Virol, 1977. **36**(1): p. 59-74.
8. Cheng, S.B., et al., *Anatomical location and redistribution of G protein-coupled estrogen receptor-1 during the estrus cycle in mouse kidney and specific binding to estrogens but not aldosterone.* Mol Cell Endocrinol, 2014. **382**(2): p. 950-9.
9. Botta, A., et al., *Erythrocytes and cell line-based assays to evaluate the cytoprotective activity of antioxidant components obtained from natural sources.* Toxicol In Vitro, 2014. **28**(1): p. 120-4.
10. Boukamp, P., et al., *Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line.* J Cell Biol, 1988. **106**(3): p. 761-71.
11. Li, Z., et al., *New insights into the pharmacological chaperone activity of c2-substituted glucoimidazoles for the treatment of Gaucher disease.* Chembiochem, 2013. **14**(10): p. 1239-47.
12. Fang, F., A.P. Wang, and S.F. Yang, *Antitumor activity of a novel recombinant mutant human tumor necrosis factor-related apoptosis-inducing ligand.* Acta Pharmacol Sin, 2005. **26**(11): p. 1373-81.
13. Takahashi, K., et al., *Spontaneous transformation and immortalization of human endothelial cells.* In Vitro Cell Dev Biol, 1990. **26**(3 Pt 1): p. 265-74.
14. Yao, Z., et al., *Enzymatic preparation of kappa-carrageenan oligosaccharides and their anti-angiogenic activity.* Carbohydr Polym, 2014. **101**: p. 359-67.
15. Drexler, H.G., et al., *Bladder carcinoma cell line ECV304 is not a model system for endothelial cells.* In Vitro Cell Dev Biol Anim, 2002. **38**(4): p. 185-6; author reply 187.
16. Jones, R.A., et al., *The role of TG2 in ECV304-related vasculogenic mimicry.* Amino Acids, 2013. **44**(1): p. 89-101.
17. van Oost, B.A., et al., *Isolation of a human von Willebrand factor cDNA from the hybrid endothelial cell line EA.hy926.* Biochem Cell Biol, 1986. **64**(7): p. 699-705.
18. Wang, J., et al., *Cytocompatibility of a silk fibroin tubular scaffold.* Mater Sci Eng C Mater Biol Appl, 2014. **34**: p. 429-36.

Supplementary Video Legends

Movie S1. Injectability of the sericin hydrogel
The crosslinked sericin hydrogels (2%, w/v, light yellow color) were injected through the needles with three different sizes, 16 G (left), 22 G (middle), and 25 G (right).

Movie S2. Elasticity of the sericin hydrogel
The crosslinked sericin hydrogel (2%, w/v, light yellow color) was placed on the top of a white plunger of a 10-ml syringe. The hydrogel was compressed vertically by another plunger. Once the compressing plunger was removed, the hydrogel restored its original shape. The white brackets indicate the height of the hydrogel before, during and after compression.