Supporting Information

Theragenerative Injectable Bone-Adhesive Hydrogels for Combined Photothermal Osteosarcoma Therapy and Bone Repair

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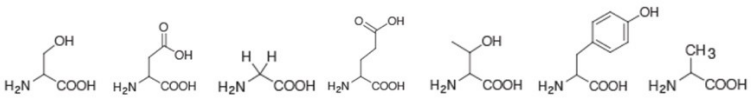
Chemical and Microstructural Characterizations: For chemical and microstructure characterization, the precursors were measured after drying, while the hydrogels were tested after freeze-casting in liquid nitrogen and dried by supercritical CO_2 drying method to obtain the corresponding aerogel. For ^1H nuclear magnetic resonance (^1H NMR) spectra of precursors (SF, SS, SSDopa and dopamine monomer), 0.005 g dry sample was dissolved in deuterium oxide (D_2O, 0.5 mL) and analyzed on a Bruker Avance II 300 MHz spectrometer. The molecular structure with main functional groups of SF, SS, SFO and final aerogels were characterized by Fourier-transform infrared (FT-IR) spectra using a Spectrum 400 (PerkinElmer) at room temperature in the wavenumber range of 400- 4000 cm^{-1}. The successful oxidation of SF to SFO was characterized by the change in absorption peaks of the 15x diluted solutions against DI water in UV-Vis spectra recorded by a PerkinElmer Lambda 950 at room temperature. The porous microstructure of the final aerogels were observed by scanning electron microscopy (SEM) on a Zeiss Neon40 CrossBeam machine. The aerogels were cut horizontally and vertically presenting different morphologies, which were examined after being treated with gold sputtering with 10 nm thickness. Energy dispersive X-ray spectroscopy (EDX) is acquired simultaneously during SEM testing as the machine is equipped with Oxford's INCA system for SEM-EDX images.

Preparation of Commercial Aqueous SF and SS Solution: Commercially available SF and SS solutions (Fibrothelium GmbH, Aachen, Germany) were used as a standard for the comparison of the extracted proteins. Specifically, SF and SS aqueous solutions were obtained using PureSilk® technology, enabling medical-grade quality production of silk on an industrial scale for a broad range of concentrations. Briefly, the two proteins were separated by degumming in a hot alkali solution. Fibroin was then dissolved in a proprietary non-toxic solvent system based on Ajisawa's reagent, purified and fully dialyzed against VE water within 8 hours using tailored extraction processing. The obtained fibroin solution was then diluted to 3.5 wt% and stored at 4°C. After the degumming process, sericin was purified, dialyzed against VE water and lyophilized.

Table S1. Amino acid composition of SF and SS. Calculated according to references.[1,2]

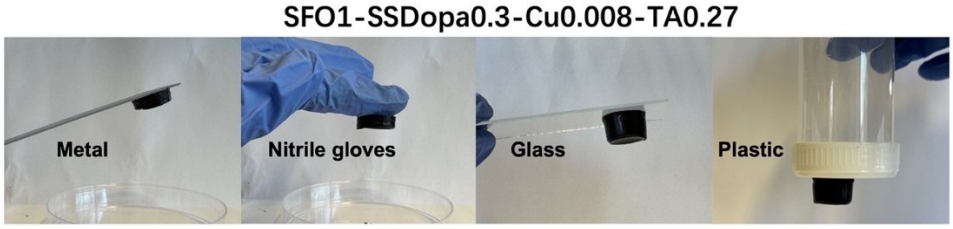


	Ser	Asp	Gly	Glu	Thr	Tyr	Ala
SF	12%	4%	35%	2%	1%	10%	30%
SS	30%	20%	11%	6%	9%	5%	4%

Table S2. Hydrogel adhesives SFO-SSDopa-Cu-TA with different recipes developed in this study.

Sample	SFO (%)	SSDopa (%)	Cu^{2+} (mol/L)	TA (%)
0	2.18	0.00	0.000	0.00
1	2.18	0.33	0.000	0.00
2	2.16	0.32	0.008	0.00
3	2.19	0.33	0.000	0.27
4	2.14	0.32	0.008	0.27
5	2.09	0.31	0.016	0.26
6	2.13	0.32	0.008	0.53
7	2.13	0.32	0.008	0.80
8	1.50	0.64	0.008	0.27
9	2.80	0.00	0.008	0.27

Some small differences in concentration of the same substance (e.g. SFO, 2.18% and 2.19%) are due to the disparity in the amount of KOH added for pH adjusting, resulting in a difference of some microliters in the volume of solution for the whole system.



Composition in the hydrogel system				Adhesive on different objects				mechanical propertiy (soft/hard)
SFO (%)	SSDopa (%)	Cu (mol/L)	TA (%)	Metal	Nitrile gloves	Glass	Plastic	
2.14	0.32	0.008	0.27	√ √	√	√ √	√ √	normal
2.09	0.31	0.016	0.26	√	×	√	√	normal-hard
2.04	0.31	0.023	0.25	√	×	√	√	hard
1.99	0.30	0.030	0.25	√	×	×	√	hard
1.50	0.64	0.008	0.27	√ √	√ √	√ √	√ √	soft-normal
2.14	0.32	0.008	0.27	√ √	√	√ √	√ √	normal
2.79	0.00	0.008	0.27	√	×	√	√	normal-hard
2.14	0.32	0.008	0.27	√ √	√	√ √	√ √	normal
2.13	0.32	0.008	0.53	√ √	×	√	√	normal
2.13	0.32	0.008	0.80	√	×	×	√	normal-hard

Figure S1. Subjective evaluation of the adhesion of hydrogel with different components to common materials (metal, nitrile gloves, glass and plastics) and their mechanical properties (hard or soft).

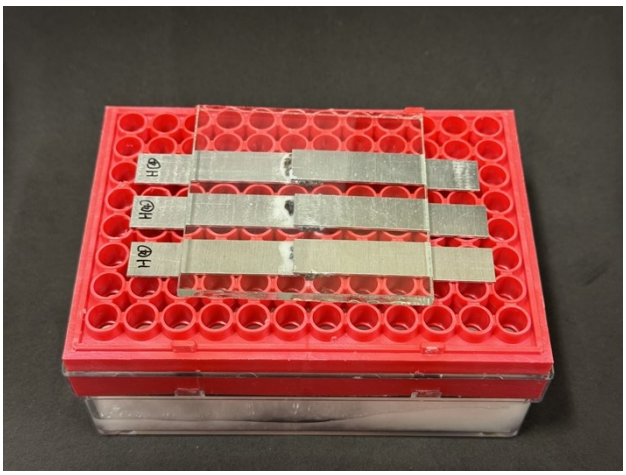


Figure S2. Preparation of samples for hydrogel adhesion tensile testing with 50 g glass fitting the joints.

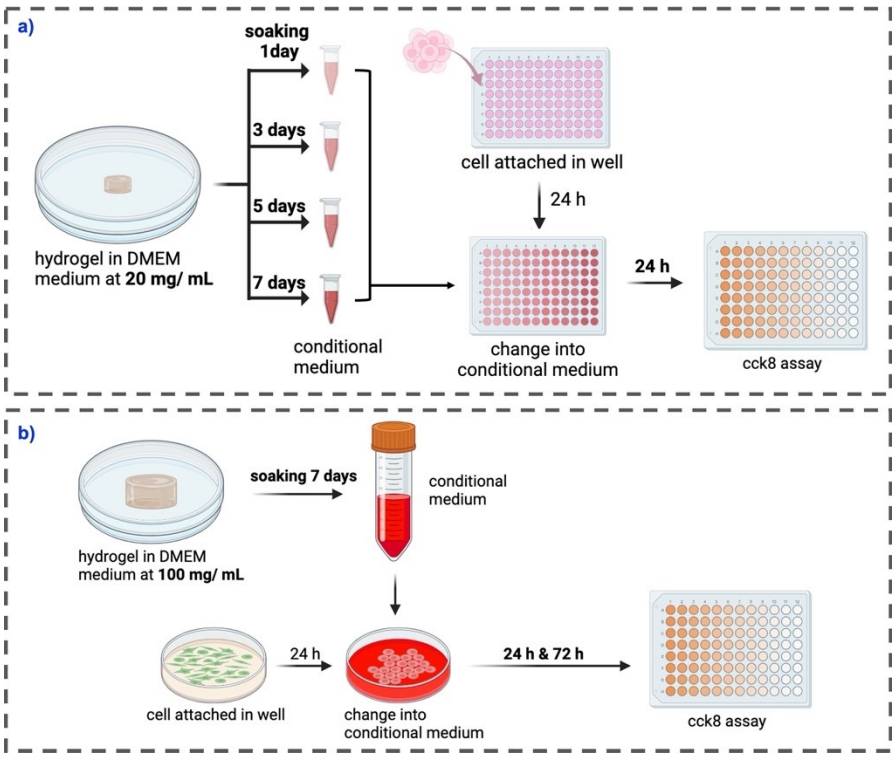


Figure S3. Indirect assessment of cell viability and proliferation for a) conditional medium after 1, 3, 5, and 7 days of soaking in low concentration (20 mg/ mL) hydrogel, and b) conditional medium after 7 days of soaking in high concentration (100 mg/ mL) hydrogel.

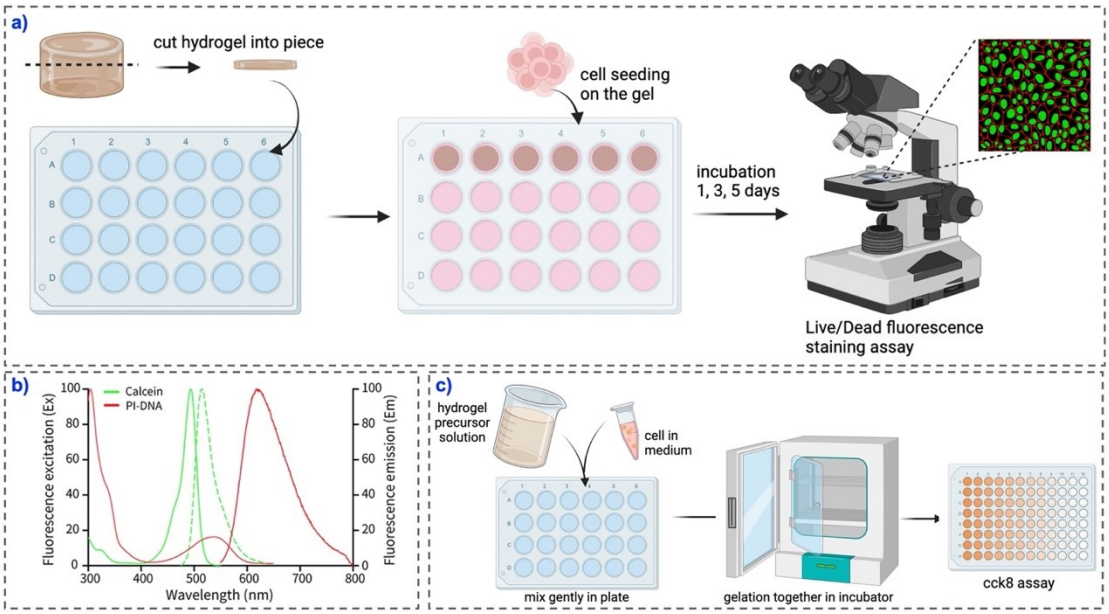


Figure S4. a) Live/Dead fluorescence staining assay for the attachment and proliferation after 2D direct cell culture. b) excitation and emission spectra of Calcein AM and PI. Adapted from *Beyotime*.[3] c) 3D culture system of hydrogels and cells.

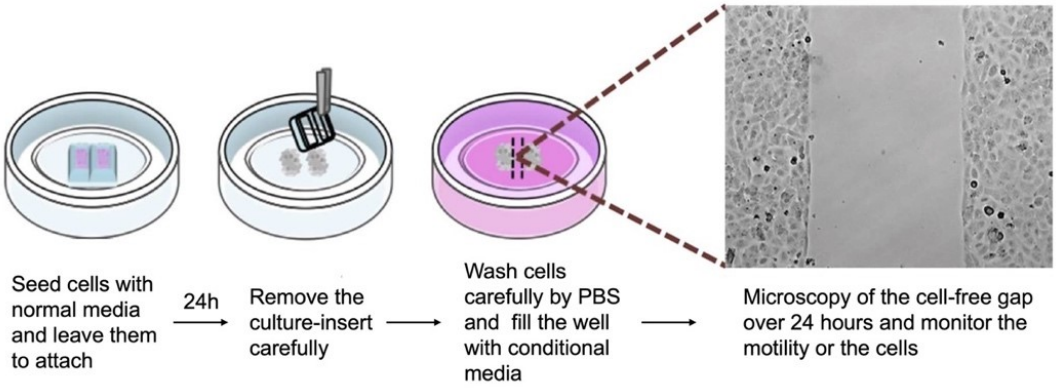


Figure S5. Schematic diagram of cell migration assay using culture inserts. Adapted from *Pijuan*.[4]

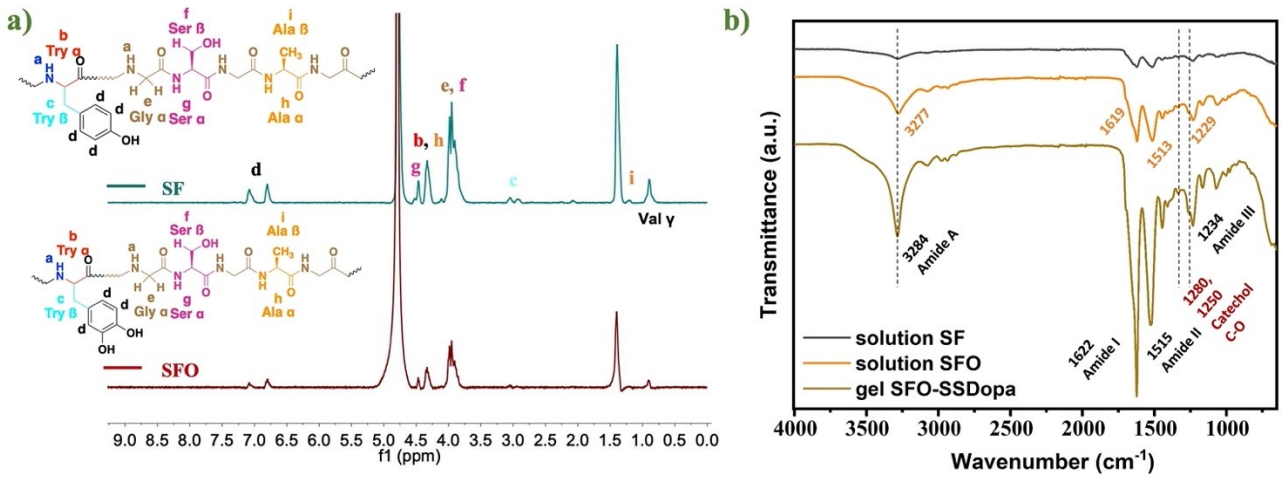


Figure S6. a) NMR spectra of SF and SFO after 1 day of oxidization and b) IR spectra of solutions SF, SFO and gel SFO-SSDopa.

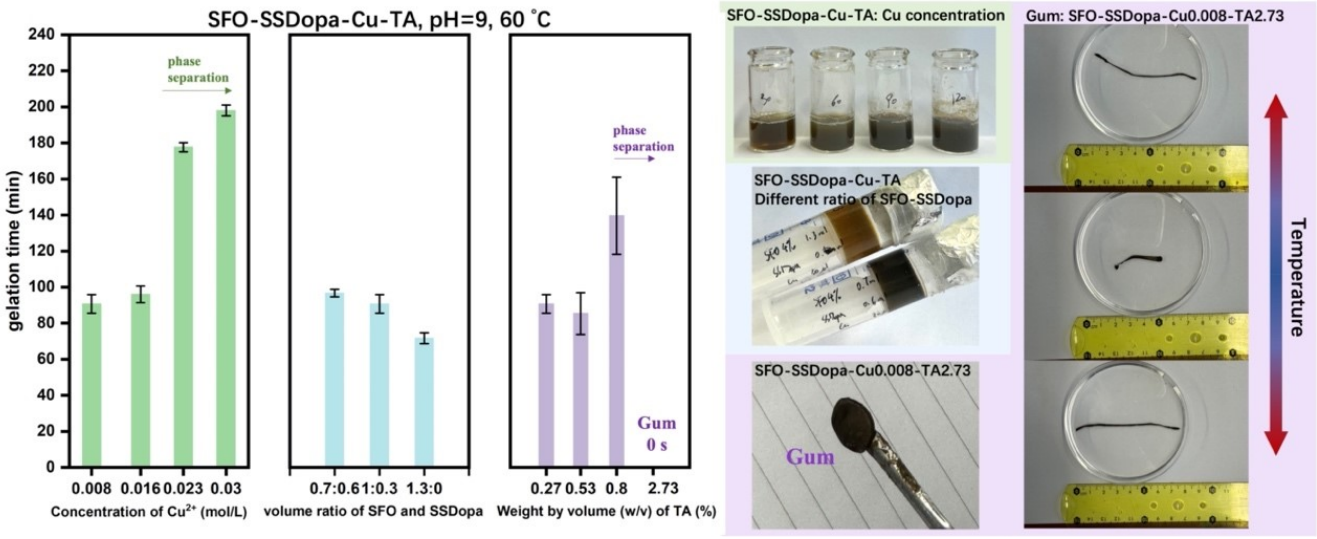


Figure S7. Effect of different parameters (concentration of Cu^{2+}, volume ratio of SFO and SSDopa, TA%) on the gelation time and the corresponding digital camera images of the obtained products. Higher concentration of Cu^{2+} and TA lead to phase separation due to high local concentration. When the concentration of TA reaches a certain critical value (2.73%), what appears is no longer coagulation, but an instantaneous formation of gum. The volume ratio of SFO and SSDopa changed the texture and color of hydrogels.

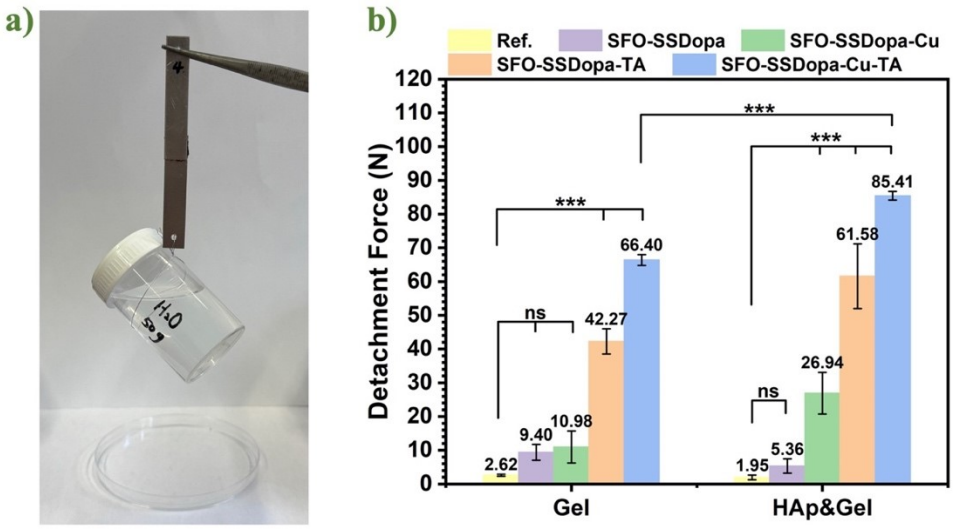


Figure S8. a) Sample lifted from a 50 g water bottle, showing a strong adhesive effect. b) Detachment force of hydrogel samples with different formulations compared to the control group (Reference sample with only Gel or Hap&Gel coating on metal slides). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ indicate the statistical significance between compared groups. n = 3 replicates.

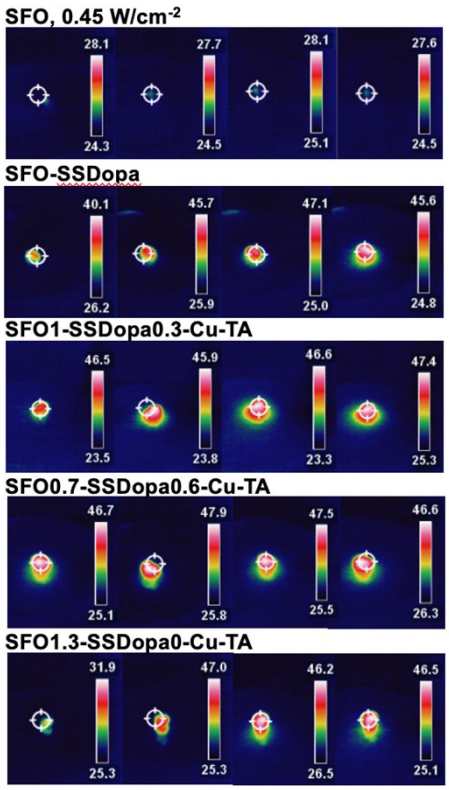


Figure S9. Digital images from a thermal camera showing the surface temperature of the sample under various power densities for assessment of the PTT efficacy of hydrogel samples with different ratios of SFO/SSDopa irradiated by 808 nm laser with 0.45 W•cm^{-2} power densities in the dry environment.

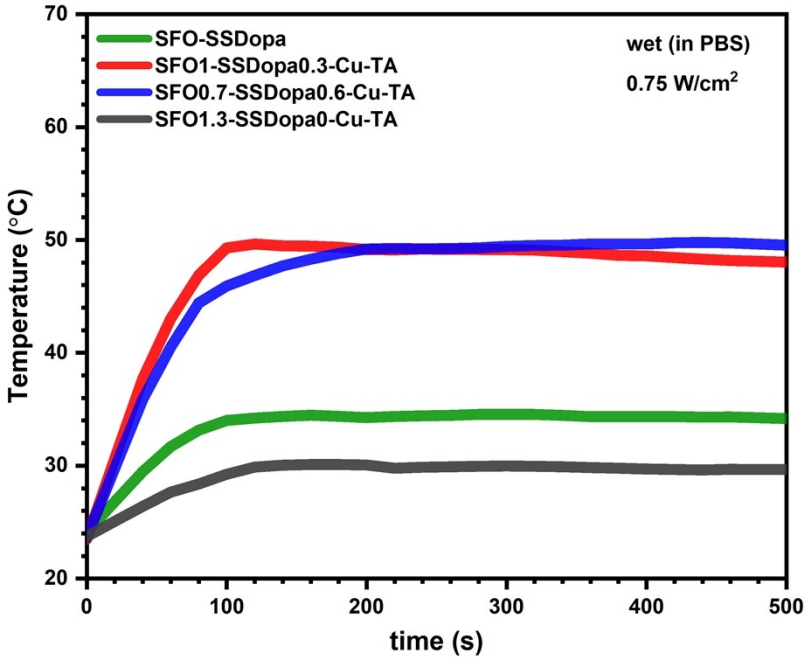


Figure S10. PTT effect of hydrogel samples with different ratios of SFO/SSDopa at 0.75 W•cm^{-2} power densities in PBS.

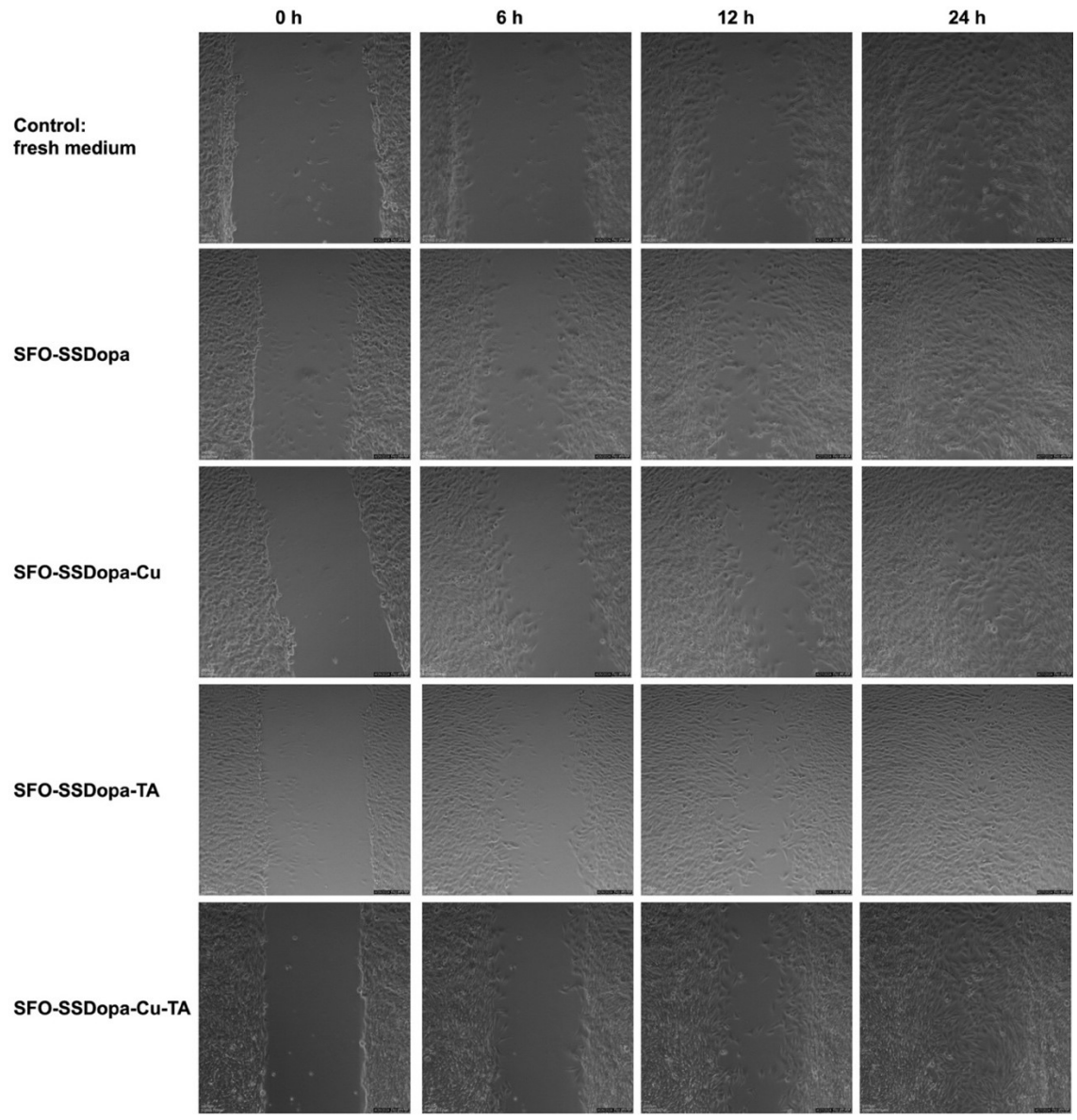


Figure S11. Dynamic monitoring images (0, 6, 12 and 24 h) of migration of MC3T3-E1 cell in the fresh medium as control and hydrogel extracts (20 mg/ mL, collected after 5 days soaking) of different components.

Reference:

(1) Zhang, Y.; Sheng, R.; Chen, J.; Wang, H.; Zhu, Y.; Cao, Z.; Zhao, X.; Wang, Z.; Liu, C.; Chen, Z.; Zhang, P.; Kuang, B.; Zheng, H.; Shen, C.; Yao, Q.; Zhang, W. Silk Fibroin and Sericin Differentially Potentiate the Paracrine and Regenerative Functions of Stem Cells Through Multiomics Analysis. *Advanced Materials* **2023**, *35* (20), 2210517. https://doi.org/10.1002/adma.202210517.

(2) Sahoo, J. K.; Hasturk, O.; Falcucci, T.; Kaplan, D. L. Silk Chemistry and Biomedical Material Designs. *Nat Rev Chem* **2023**, *7* (5), 302–318. https://doi.org/10.1038/s41570-023-00486-x.

(3) Beyotime. *Calcein/PI Cell Activity and Cytotoxicity Assay Kit*. https://www.beyotime.com/product/C2015L.htm (accessed 2024-04-23).
(4) Pijuan, J.; Barceló, C.; Moreno, D. F.; Maiques, O.; Sisó, P.; Marti, R. M.; Macià, A.; Panosa, A. In Vitro Cell Migration, Invasion, and Adhesion Assays: From Cell Imaging to Data Analysis. *Front. Cell Dev. Biol.* **2019**, *7*. https://doi.org/10.3389/fcell.2019.00107.