

Supporting Information

Design of Self-Healing and Electrically Conductive Silk Fibroin-Based Hydrogels

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Table S1. Amino acid contents of the regenerated silk fibroin (SF).

Amino acids	Mol (%)	Amino acids	Mol (%)	Amino acids	Mol (%)
CySO₃H	0.005±0.002	Cys	2.529±0.303	Thr-ol	0.637±0.001
Tau	0.001±0.000	Val	2.728±0.556	Lys	0.218±0.005
Asp	1.378±0.022	Met	0.388±0.271	NH₃	2.030±0.186
Thr	0.792±0.015	Ile	0.645±0.036	His	0.247±0.033
Ser	8.637±0.224	Leu	0.396±0.022	Trp	0.001±0.001
Glu	1.794±0.216	Tyr	4.493±0.019	Arg	0.336±0.003
Gly	42.390±0.064	Phe	0.712±0.046	Hypro	0.022±0.031
Ala	29.154±1.031	g-ABA	0.000±0.000	Pro	0.470±0.015

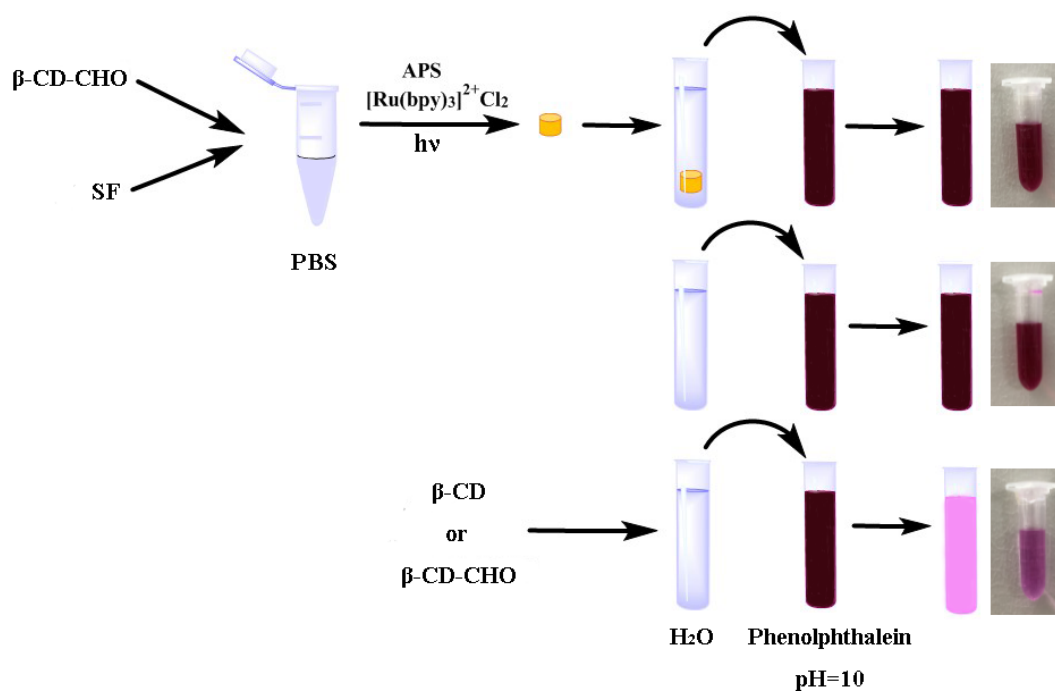


Figure S1. Conjugation of β -cyclodextrin (β -CD) on to SF. Non observable color change of the phenolphthalein colorimetric solution implied that β -CD was grafted on SF without releasing any β -CD from the hydrogels.

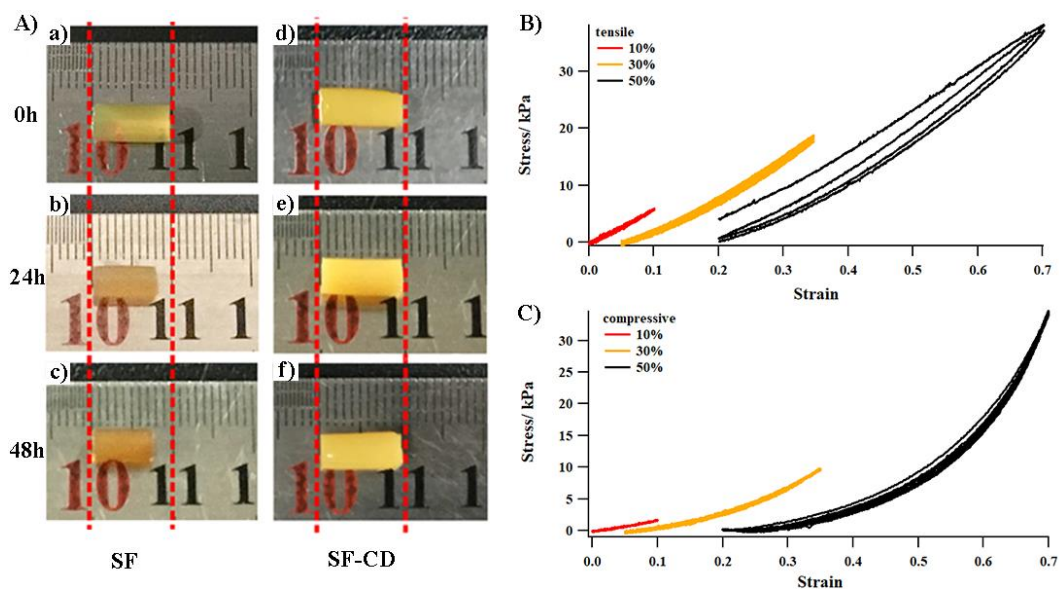


Figure S2. Formation of SF-CD dual-cross-linked hydrogels. A) Swelling profile of SF-based chemically cross-linked hydrogels (a-c) and SF-CD dual-cross-linked hydrogels (d-f). Representative stress-strain curves obtained from B) tensile and C) compressive mechanical tests on $100 \text{ mg} \cdot \text{ml}^{-1}$ SF-CD dual-cross-linked hydrogels.

In order to further tailor the structure and performance of SF-CD dual-cross-linked hydrogels, we grafted azobenzene (Azo) groups onto SF (SF-Azo) using a diazonium coupling chemical reaction targeting Tyr residues ¹ (Figure S3A). The extent of modification using an aniline derivative and the product was characterized using FTIR and UV/Vis spectroscopies. Peaks at 2100 cm^{-1} and (1080 , 830 and 520 cm^{-1}) were assigned to $\text{-C}\equiv\text{C}$ and benzene, respectively. A new signal at 2270 cm^{-1} appeared in FTIR, indicating formation of $\text{-N}^+\equiv\text{N}$ (Figure S3B). A strong absorption at 340 nm appeared in UV/Vis, corresponding to the p-p^* transition of the newly formed Azo group on SF (Figure S3C). A dual-cross-linked hydrogel could be obtained based on SF-CD and SF-Azo (Figure S3DE). The (SF-CD + SF-Azo) dual-cross-linked hydrogels exhibited improved electrochemical performance (Figure S3FG). It is interesting to notice that color of the (SF-CD + SF-Azo) dual-cross-linked hydrogels turned red upon a charge, and that the color change was reversible (Figure S3H). This observation could be explained by electric field-induced isomerization of azobenzene ². In addition, the choice of 4-ethynylaniline in the present study would allow for secondary reactions to introduce a wide variety of new functional groups into SF using click chemistry ³.

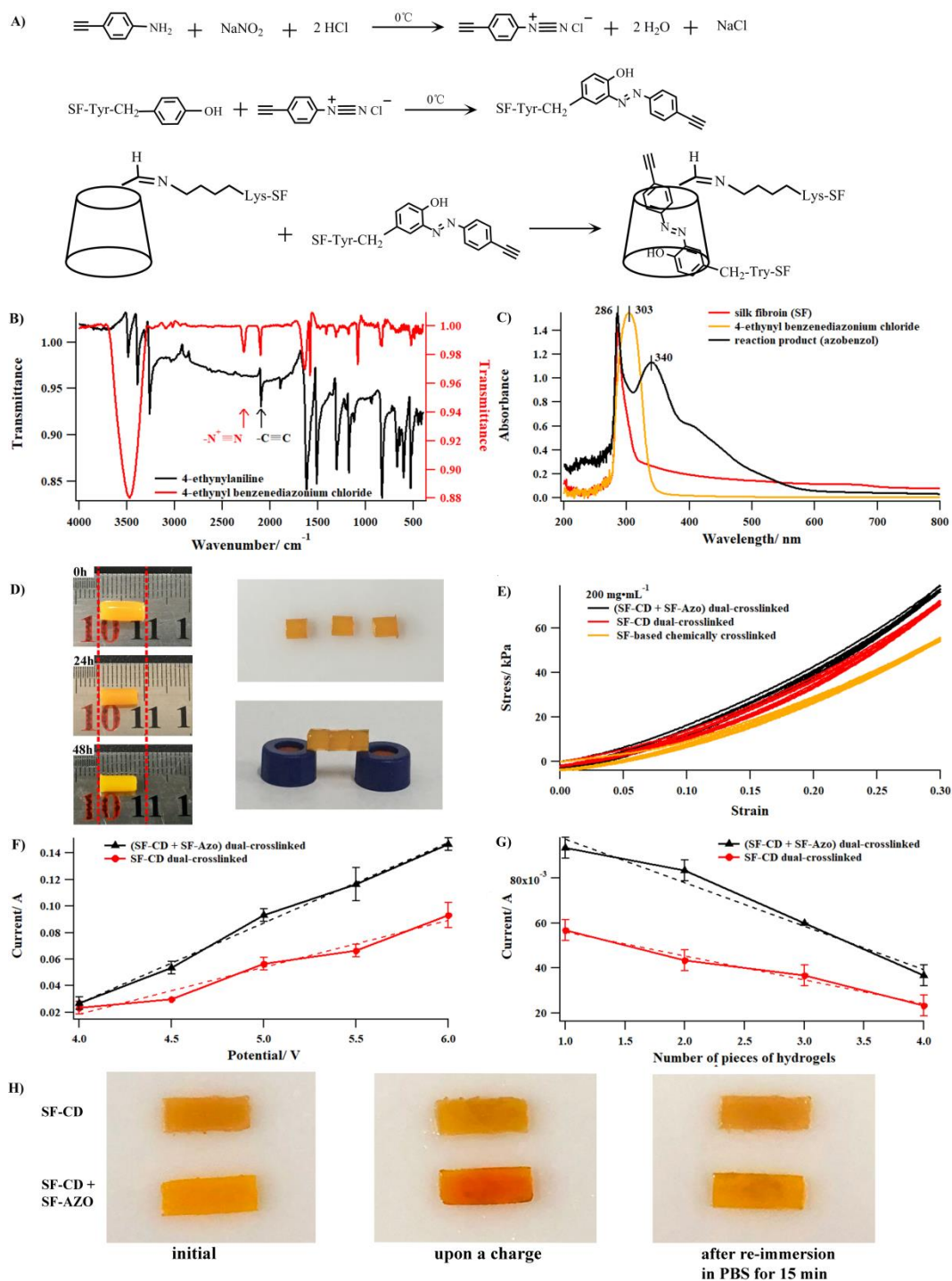


Figure S3. Modification of SF with azobenzene (Azo) groups. A) Schemes of diazonium coupling reaction with Tyr residues on SF, and non-covalent host-guest interactions between SF-CD and SF-Azo. B) Confirmation of $-N^+\equiv N$ by a new peak at 2270 cm^{-1} in FTIR spectrum.

C) Comparison of the UV/Vis spectra of unmodified and modified SF in deionized water at pH 7 confirmed Azo groups as revealed by appearance of a strong absorption at 340 nm. D) Swelling profile of dual-cross-linked (SF-CD + SF-Azo) hydrogel samples, sliced samples, a self-healed sample after rejoining the sliced samples. E) Representative stress-strain curves obtained from compressive mechanical tests on SF-based chemically cross-linked hydrogels, SF-CD dual-cross-linked hydrogels and (SF-CD + SF-Azo) dual-cross-linked hydrogels. F-H) Electrical performance of the SF-CD dual-cross-linked hydrogels in 0.25 M Na₂SO₄. Change of current as F) applied potential and G) thickness of adhered hydrogels increased. H) Reversible change of the (SF-CD + SF-Azo) dual-cross-linked hydrogel color during electrochemical tests.

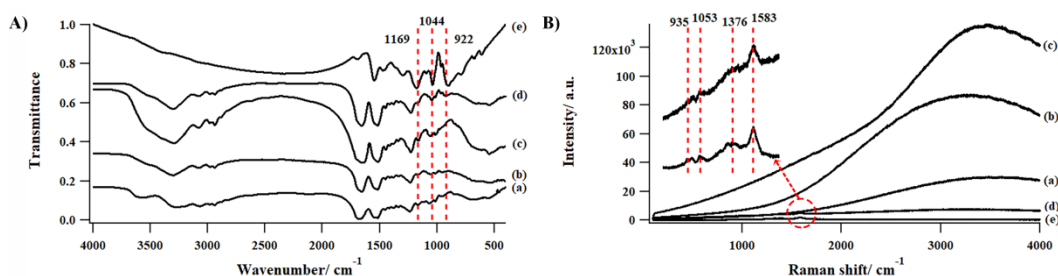


Figure S4. Chemical structure of the hydrogels. A) FTIR spectra. Appearance of peaks associated to PPy were identified at 1169, 1044 and 880 cm⁻¹, attributed to the bending and stretching of the amine groups. B) Raman spectra. Peaks associated to PPy were identified at 935, 1053, 1376 and 1586 cm⁻¹. The broad peaks at ~ 3000 cm⁻¹ were characteristic peaks of SF-based hydrogels. (a) SF-based chemically cross-linked hydrogels, (b) SF-CD dual-cross-linked hydrogels, PPy-coated SF-CD dual-cross-linked hydrogels prepared by the (c) enzymatic and (d) chemical methods, (e) PPy.

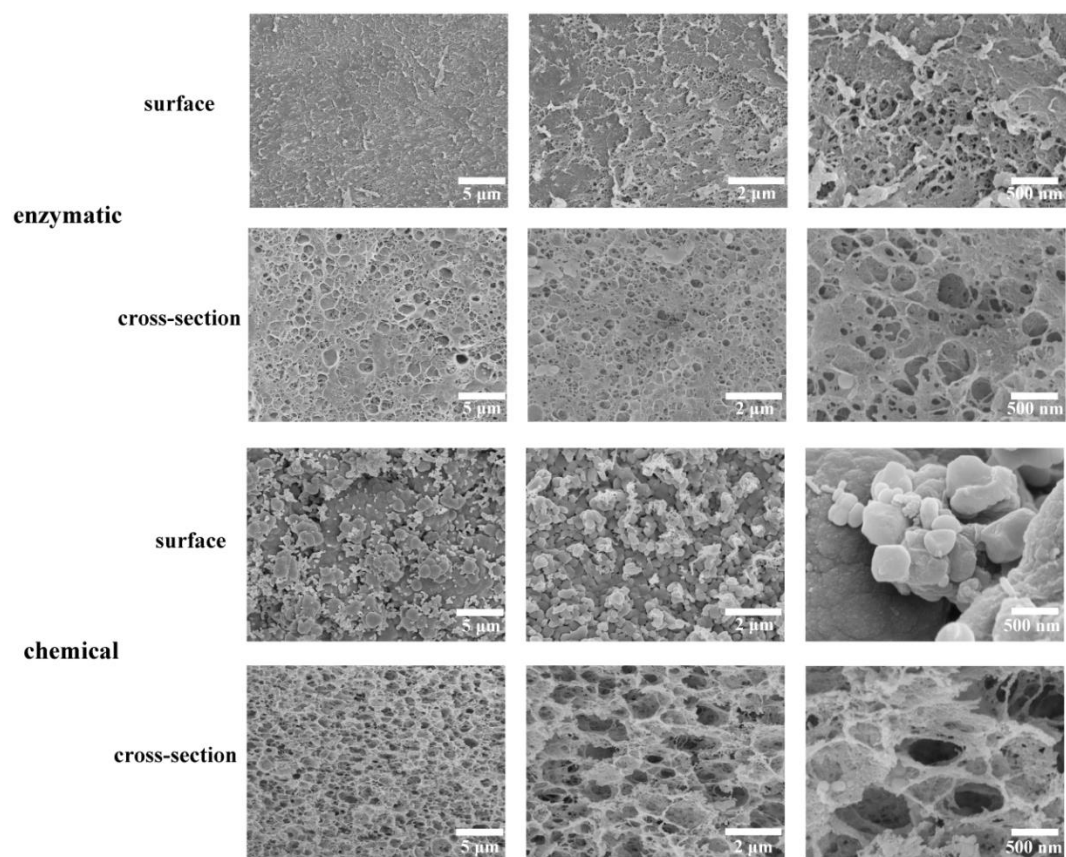


Figure S5. SEM images of freeze dried scaffolds of the PPy-coated SF-CD dual-cross-linked hydrogels. The scaffolds prepared via the enzymatic polymerization method were covered by layers of PPy, while particles of PPy were coated on the scaffolds prepared via the chemical polymerization method.

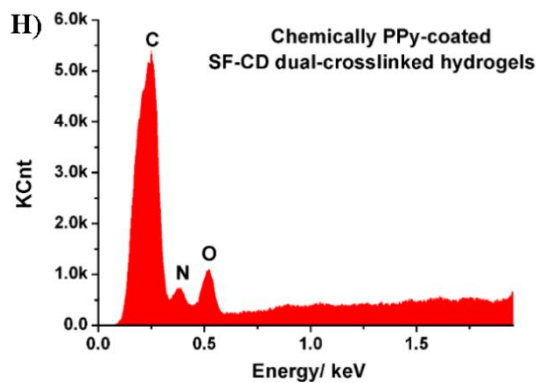
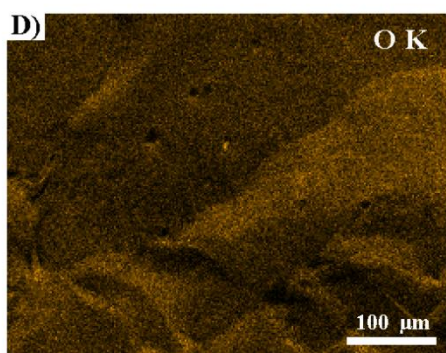
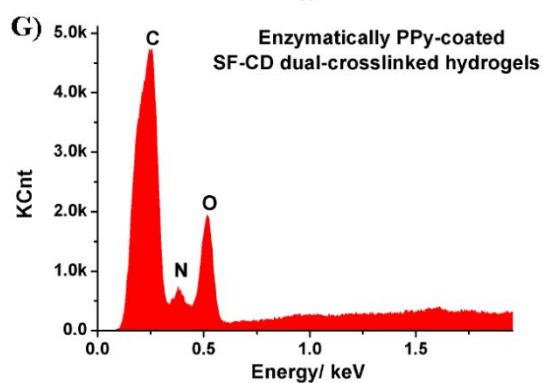
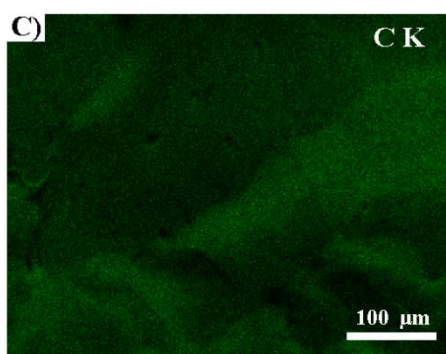
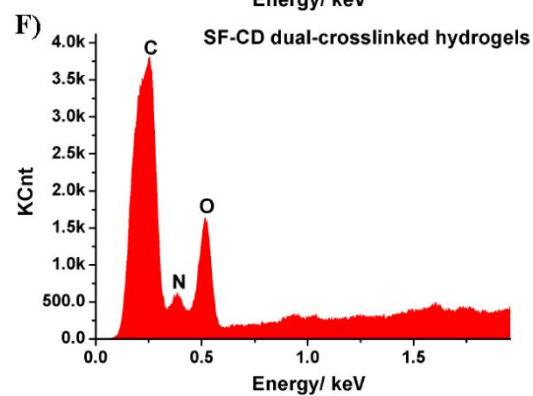
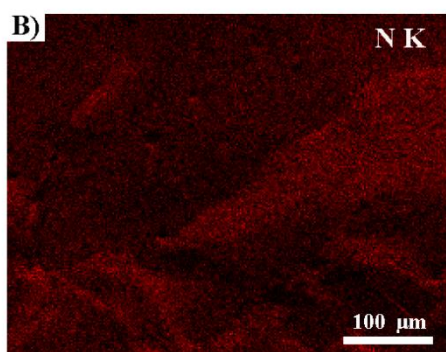
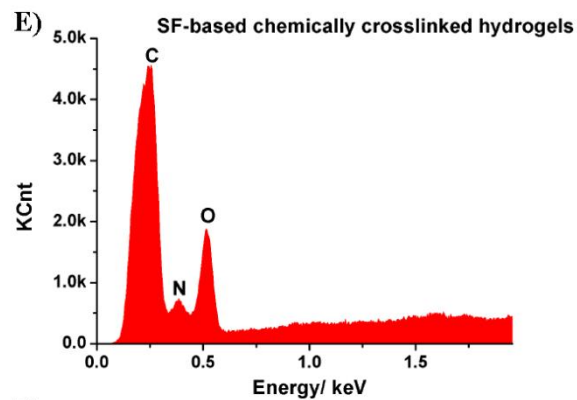
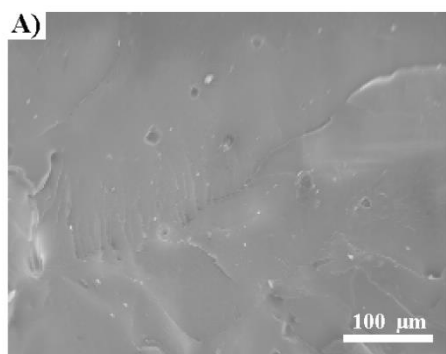


Figure S6. EDX mapping. A-D) Representative images of PPy-coated SF-CD dual-cross-linked hydrogels prepared by the enzymatic method showed original, nitrogen atom based, carbon atom based and oxygen atom based images, respectively. E-H) EDX analysis of the SF-based chemically cross-linked hydrogels, SF-CD dual-cross-linked hydrogels, PPy-coated SF-CD dual-cross-linked hydrogels prepared by the chemical and enzymatic methods, respectively.

Table S2. Element weight percentage (wt%) determined by EDX analysis.

Samples	C/ wt%	N/ wt%	O/ wt%
SF-based chemically cross-linked hydrogels	49.73	23.64	26.64
SF-CD dual-cross-linked hydrogels	50.18	22.02	27.81
Enzymatically PPy-coated SF-CD dual-cross-linked hydrogels	52.41	20.60	26.98
Chemically PPy-coated SF-CD dual-cross-linked hydrogels	62.13	20.71	17.16
Pyrrole ^a	72	21	-

^a Theoretical values based on atomic and molecular weight.

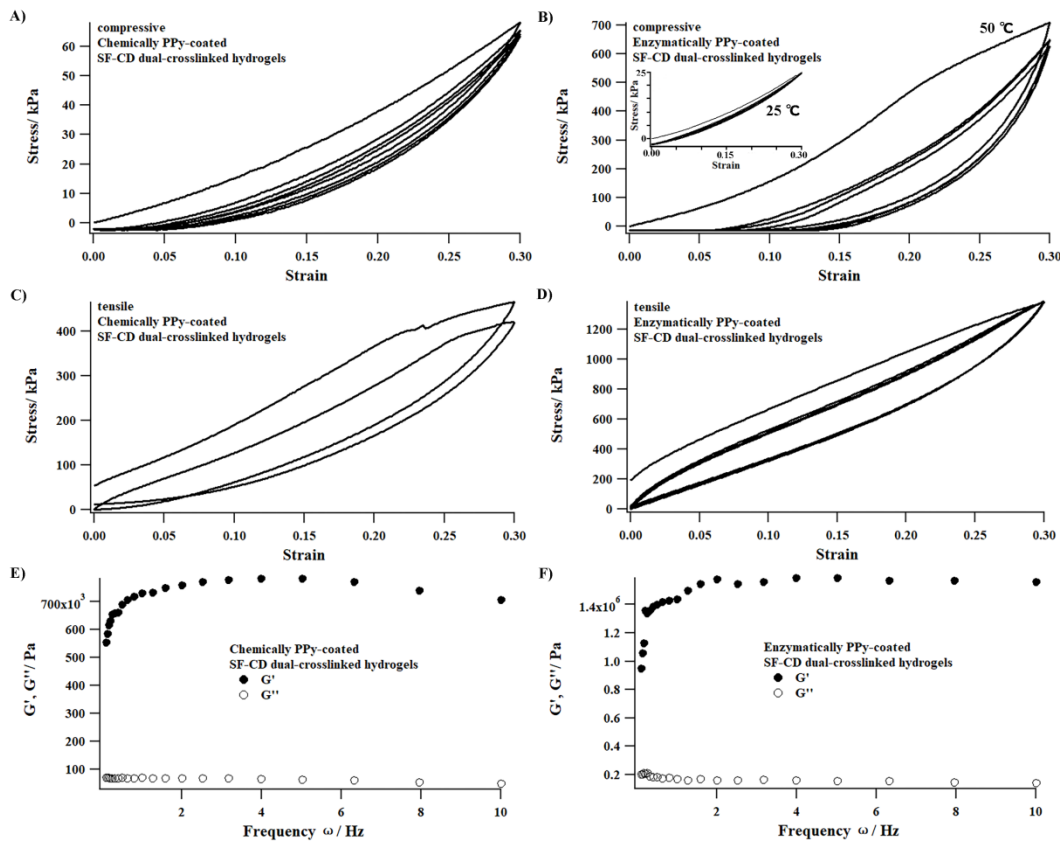


Figure S7. Mechanical properties of the PPy-coated SF-based hydrogels. Representative stress-strain curves obtained from compression and tensile tests on the hydrogels prepared by the AC) chemical and BD) enzymatic methods. Frequency dependence of the storage modulus (G') and loss modulus (G'') for the hydrogels prepared by the E) chemical and F) enzymatic methods.

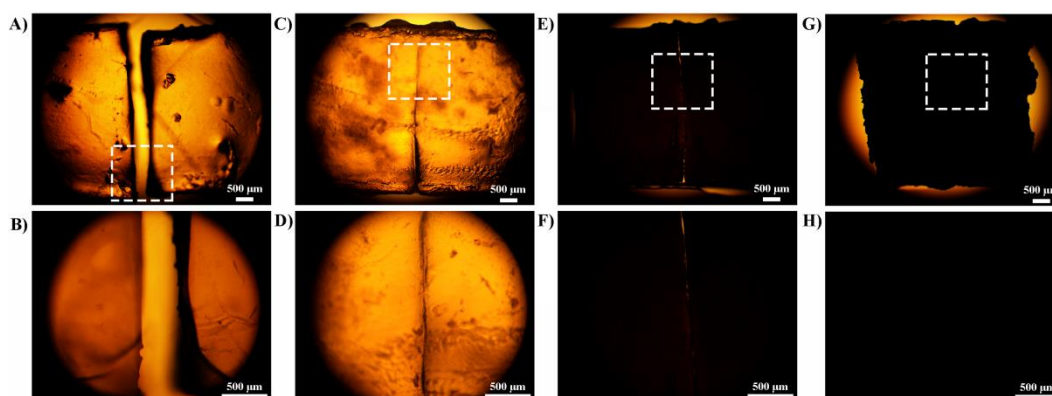


Figure S8. Optical microscopic images of interface between hydrogel pieces. Self-healing behavior of the hydrogels was analyzed by measuring the disappearance of the gap between two interactive surfaces. AB) SF-based chemically cross-linked hydrogels showed no recovery, CD) SF-CD dual-cross-linked hydrogels showed $\sim 86.7\%$ recovery, EF) PPy-coated SF-CD dual-cross-linked hydrogels prepared by the enzymatic method showed $\sim 81.7\%$ recovery, and GH) PPy-coated SF-CD dual-cross-linked hydrogels prepared by the chemical method showed $\sim 97.3\%$ recovery.

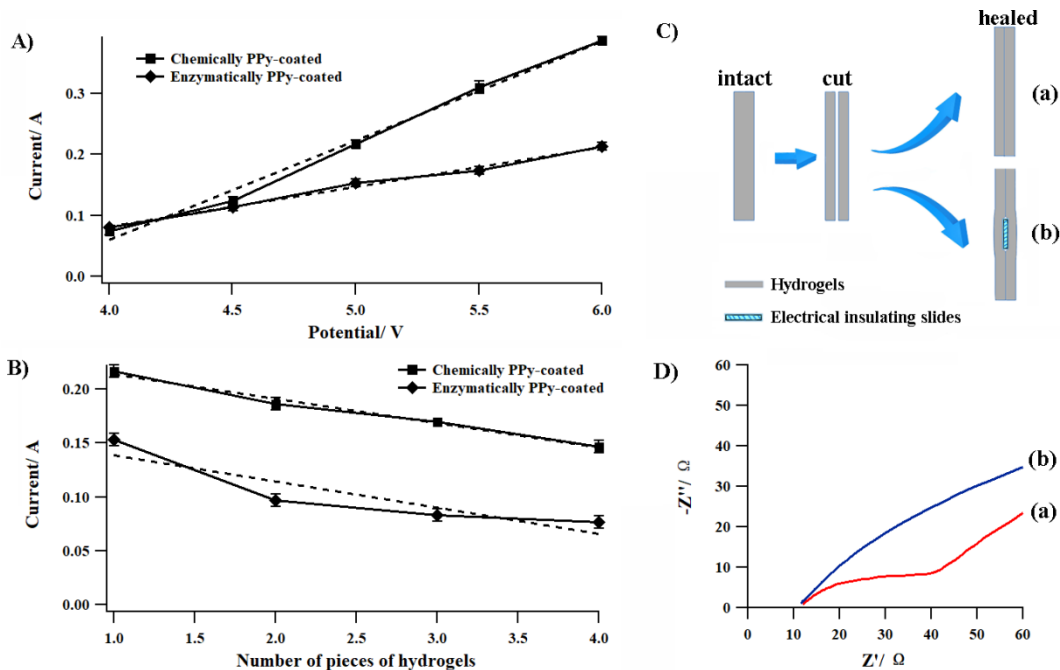


Figure S9. Electrochemical performance of the PPy-coated SF-CD dual-cross-linked hydrogels.

Change of current as A) applied potential and B) thickness of adhered hydrogels increased during electrochemical tests in 0.25 M Na_2SO_4 . It is of note that the conductivity was attributed to combination of the electrical property of ppy and ionic mobility of the electrolyte solution in and out of the hydrogel network, as there was still detectable current even when no hydrogels were involved during the tests. C) Illustration of EIS experiments and D) representative Nyquist plots on hydrogel samples with intentional introduction of poor alignment by putting electrical insulating slides between sliced hydrogels.

Importantly for applications, the hydrogels could potentially be used as scaffolds in cell studies for biological applications. As the first step towards this goal, we evaluated cytocompatibility of the hydrogels. The L929 mouse fibroblast cell line was employed throughout the cell studies. Cytotoxicity was assessed by determining cell viability in the presence of 4 mg mL⁻¹ SF, 4 mg mL⁻¹ SF-CD, 4 mM pyrrole and 4mM PPy. The MTT assays demonstrated that the main components of the PPy-coated SF-CD dual-cross-linked hydrogels were not cytotoxic within the range of concentration tested, warranting further investigation as promising biocompatible materials for tissue engineering applications.

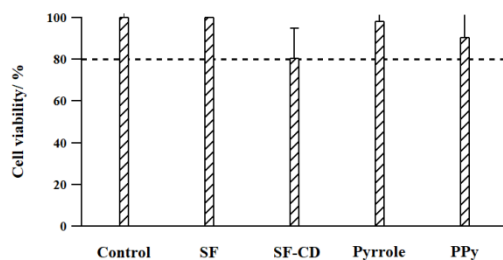


Figure S10. Cytotoxicity evaluation of main components of the PPy-coated SF-CD dual-cross-linked hydrogels. MTT assays on L929 mouse fibroblasts after 24 h of incubation showed good cell viability (>80%). Data are normalized to cell number without added reagents.

Movie S1. Self-healing properties of SF-CD dual-cross-linked hydrogels.

Movie S2. Self-healing properties of SF-CD dual-cross-linked hydrogels after deposition of PPy.

References

1. Murphy, A. R.; John, P. S.; Kaplan, D. L., Modification of Silk Fibroin Using Diazonium Coupling Chemistry and the Effects on hMSC Proliferation and Differentiation. *Biomaterials* 2008, 29, 2829-2838.
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3. Han, Y.; Lv, S., Synthesis of Chemically Crosslinked Pullulan/Gelatin-Based Extracellular Matrix-Mimetic Gels. *Int. J. Biol. Macromol.* 2019, 122, 1262-1270.