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Tannin-Bridged Magnetic Responsive Multifunctional Hydrogel for Enhanced Wound Healing by Mechanical Stimulation Induced Early Vascularization

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In vivo cytocompatibility evaluations

NIH3T3 cells ($2x10^4$) were seeded in 24-well cell culture plates containing hydrogels and cultured in magnetic (80 mT) and non-magnetic incubators, respectively. After 3 days cell culture, live-dead staining was performed to observe the cytocompatibility of the hydrogels by fluorescent inverted microscope (TE2000U, Nikon). Moreover, cells were cultured to 3 and 7 days to assess the proliferation activity of cells by CCK-8 kit (seven sea) and the absorbance at 450 nm was measured by multifunctional microplate scanner (Infinite M200, Tecan, Switzerland). HUVECs ($2x10^5$) were seeded in 24-well cell culture containing hydrogels and cultured in the same way as NIH3T3.

Tubule formation assays of HUVECs

A tubule formation assay on the Matrigel (No.354234, Corning) was used to investigate the tube-formation ability of HUVECs after cocultured with different hydrogel in the absence/presence of SMF. HUVECs were cocultured with hydrogel for 48 h, and then, the cells seeded on the hydrogels were resuspended and diluted to a density of 2×10^4. Then 100 μL of the cell suspension was added to each well of a 48-well plate coated with Matrigel. After 6 h, images were captured with an inverted microscope and analyzed using ImageJ 1.51 software.

Quantitative Real-time Polymerase Chain Reaction (PCR)

The expression of the genes of TNF-α and iNOS was quantitatively evaluated via real-time PCR. RAW264.7 cells were cultured on various hydrogel in the absence/presence of SMF for 4 days. The total RNA of the cells cultured on different hydrogel was extracted using TRIzol Reagent (Invitrogen, Thermo Fisher, USA) on the basis of the manufacturer's manual. The purity and concentration of RNA were assessed by Nanodrop Plates (Infinite M200, Tecan, Switzerland). The mRNAs of all the samples were reversely transcribed according to the description in Prime Script RT Reagent Kit with gDNA Eraser RR047A (TaKaRa, Japan). The expression of related genes was quantified using SYBR Premix Ex Taq RR420A (TaKaRa, Japan). Gene-specific primers containing glyceraldehyde-3-phosphate dehydrogenase (GAPDH), tumor necrosis factor α (TNF-α), and inducible Nitric Oxide Synthase (iNOS) were

designed by the primer design software of beacon 5.0. Real-time PCR analysis was implemented using Stratagene Mx3005P Real-time PCR System (Agilent Technologies Inc., USA) and the gene expression levels were acquired by the threshold cycles (Ct). Relative transcript quantities were calculated through using the ΔΔCt method. GAPDH was used as a reference gene and was amplified as well as the target genes from the same cDNA samples. The difference of the Ct value between the sample and GAPDH was defined as the ΔCt. The difference in the ΔCt of the cells grown on the experimental groups relative to the control group cells was defined as the ΔΔCt. The fold change in gene expression was expressed as $2^{-\Delta\Delta Ct}$.

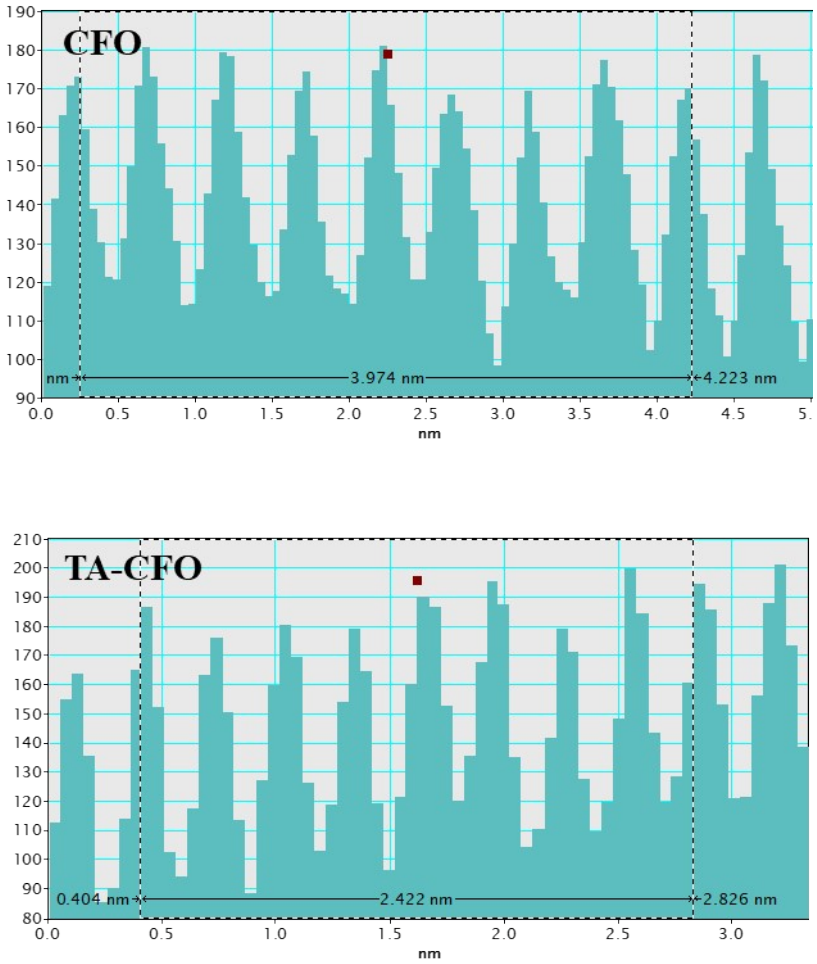

Figure S1 The line profile of the corresponding HR-TEM image of CFO and TA-CFO NPs.

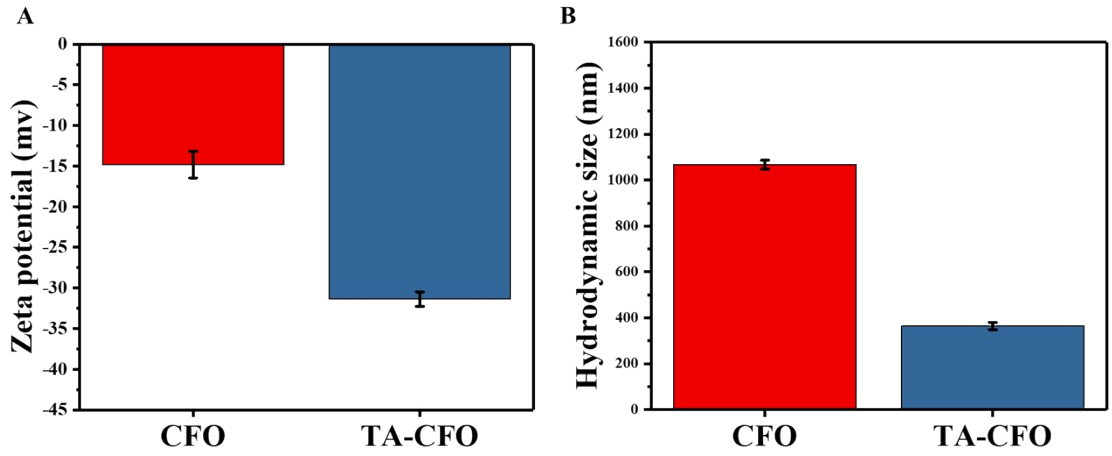

Figure S2. (A) The zeta potential of CFO and TA-CFO NPs. (B) Hydrodynamic size of CFO and TA-CFO NPs.

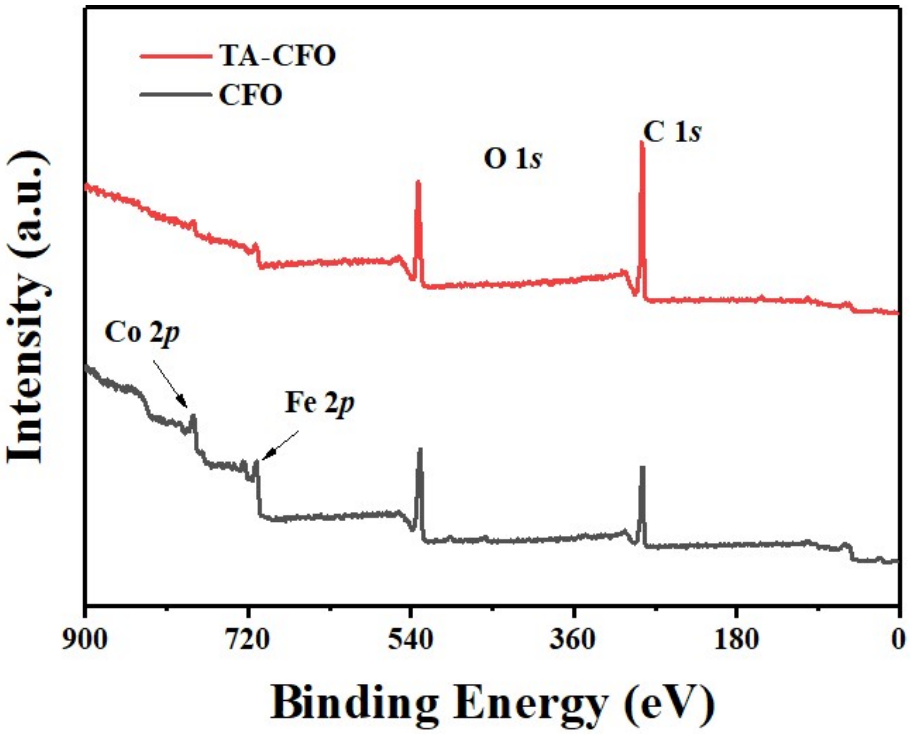

Figure S3. XPS survey scan spectra of CFO and TA-CFO NPs.

Table S1. Quantitative analysis of elements by XPS

	Name	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %
CFO	C1s	284.62	1.46	33366.53	59.71
	Fe2p3	710.72	4.27	22309.3	4.72
	Co2p3	780.09	3.91	14968.04	2.73
	O1s	530.26	2.35	48598.24	32.84
TA-CFO	C1s	284.64	1.73	61732.39	73.44
	Fe2p3	711.73	4.44	9727.55	1.37
	Co2p3	781.09	2.97	5360.15	0.65
	O1s	532.57	3.35	54587.58	24.54

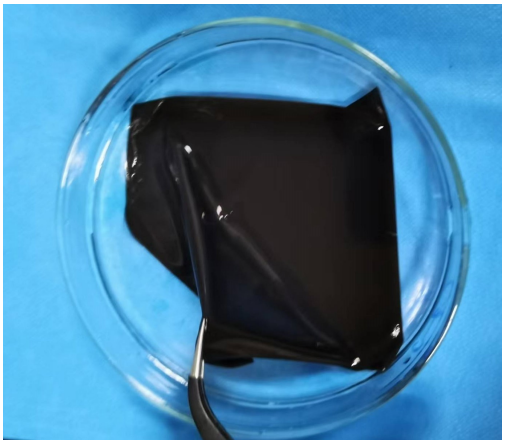

Figure S4 The gross morphology of TA-CFO/PVA magnetically responsive hydrogels.

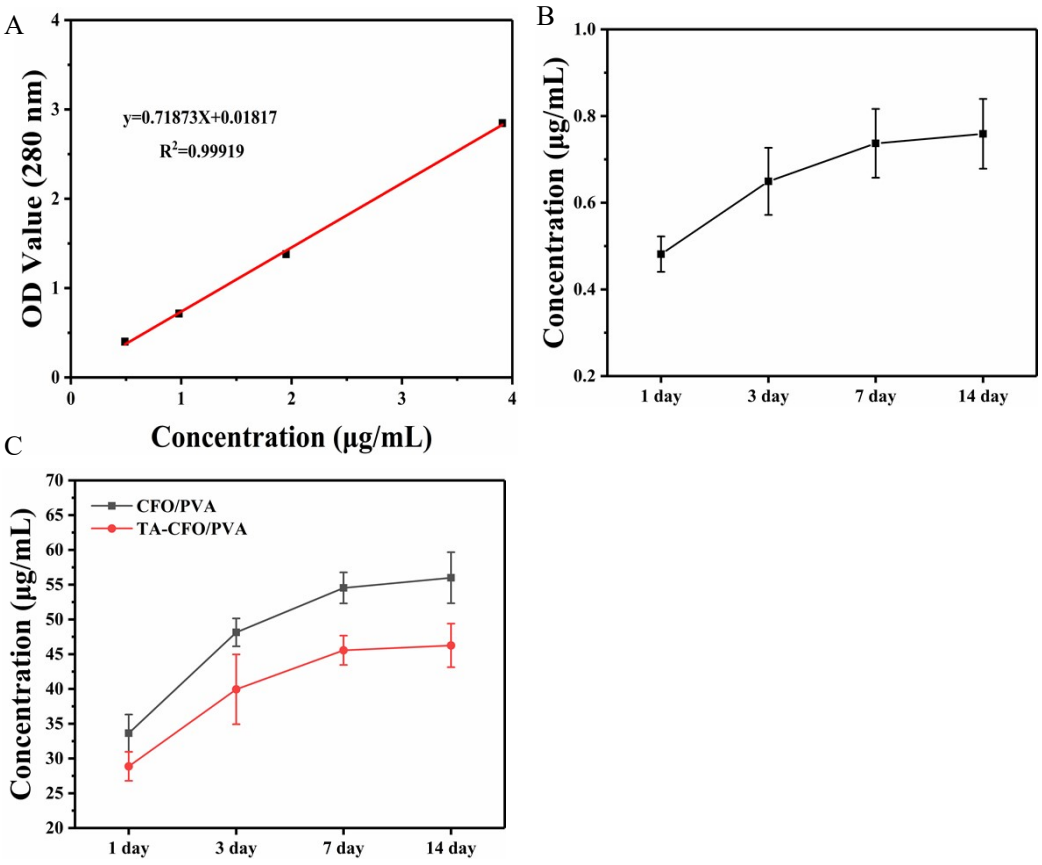

Figure S5. (A) The standard curve which was determined from the linear regression fit from the OD value (280 nm) vs TA concentration curves. (B) The release of TA from TA-CFO/PVA hydrogel. (C) The release of Co^{2+} from CFO/PVA hydrogel and TA-CFO/PVA hydrogel.

Table S2. Primers used in the RT-PCR analysis

Genes	Forward Primer	Reverse Primer
INOS	GCCCAGGAGGAGAGAGAT	GCAAAGAGGACTGTGGCT
TNF-α	CTTGTTGCCTCCTCTTTTGCTTA	CTTTATTTCTCTCAATGACCCGTAG
GAPDH	AGAAGGTGGTGAAGCAGGCATC	CGAAGGTGGAAGAGTGGGAGTTG

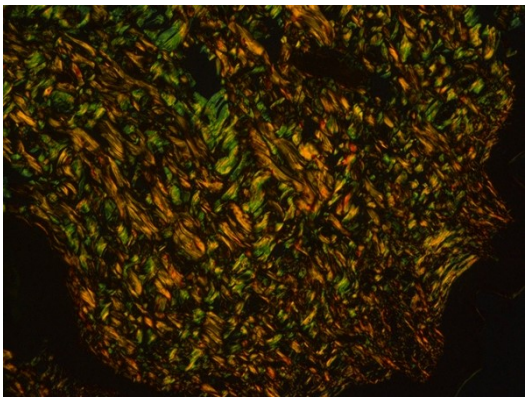

Figure S6. Sirius red staining of normal skin tissue.