Supporting information

Zeolitic Imidazolate Framework-90 loaded with methylprednisolone sodium succinate effectively reduces hypertrophic scar *in vivo*

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Experimental section

S1.1 Chemicals

$Zn(CH_3COO)_2 \cdot 2H_2O$ (98%), imidazolate-2-carboxyaldehyde (2-ICA) (99%), and dimethyl sulfoxide (DMSO) (AR, 98%) were purchased from Energy Chemical, China. Methanol (AR, 99.5%), ethanol (AR, 99.7%), and N,N-dimethylformamide (DMF) (AR, 99.5%), were purchased from the Tianjin Fuyu Fine Chemical, China. MTT solution was from Solarbio, China. Methylprednisolone sodium succinate (MPSS) was purchased from Pfizer Manufacturing Belgium NV, USA (Lot No: FC3175). In Situ Cell Death Detection Kit was bought from Roche, Switzerland.

S1.2 Quantification of MPSS@ZIF-90 loading capacity, loading efficiency, and the percentage of drug release

Drug loading was determined through HPLC assay. 15 mg MPSS@ZIF-90 was dissolved in 0.1 M hydrochloric acid, and the separation was carried out using a GL Sciences C18 column (2.1×100 mm). The mobile phase was CH_3OH/0.01 M KH_2PO_4 with a volume ratio of 65/35 and the flow rate was 0.1 mL/min. The quantification was achieved using UV detection at 254 nm (M20A photodiode array detector; Shimadzu). The loading amount in MPSS@ZIF-90 was calculated by the equation: MPSS loading capacity (wt) % = $m_{MPSS}/m_{MPSS@ZIF\text{-}90}$. The loading efficiency in MPSS@ZIF-90 was calculated by the equation: MPSS loading efficiency % = ($m_{MPSS@ZIF\text{-}90}$×MPSS loading capacity%) / ($m_{MPSS\ total\ amount\ added}$). In drug release experiments, 15 mg MPSS@ZIF-90 nanoparticles were suspended in 30 mL pH 5, 6, and 7.4 PBS respectively for 3 days. The release amount of each time point is detected by HPLC assay. The release percentages of MPSS were calculated by the equation: release percentage (%) = $m_{MPSS\ released} / m_{MPSS\ total}$.

S1.3 MTT assay protocol

The cytotoxicities of ZIF-90, MPSS, and MPSS@ZIF-90 on HHSFs were evaluated through MTT assay. HHSFs cultured in completed fibroblasts cell medium were seeded into 96-well culture plates and incubated overnight in sure of the cells' attachment. Then, fresh media with MPSS, ZIF-90 or MPSS@ZIF-90 at the desired concentration (2.5, 5, 10, 20, or 40 μg/mL) was added. After the cells were cultured for the indicated time period, 10 μL MTT solution was added to each well, and incubated for additional 4 hours.

S1.4 mRNA expression of VEGF, α-SMA, and TGFβ-1 in HHSFs and in rabbit

The primer sequences used for the *mRNA* expression of VEGF, α-SMA, and TGFβ-1 in HHSFs are as follow: GAPDH: 5′- GGGAAACTGTGGCGTGAT -3′ and 5′- GAGTGGGTGTCGCTGTTGA -3′, VEGF: 5′- CATGCAGATTATGCGGATCAA -3′ and 5′- GCATTCACATTTGTTGTGCTGTAG -3′, α-SMA: 5′- GGTGATGGTGGGAATGGG -3′ and 5′- GCAGGGTGGGATGCTCTT -3′, TGF-β1: 5′- TGCTGTGGCTACTGGTGC -3′ and 5′- CATAGATTTCGTTGTGGGTTTC -3′.

The primer sequences used for the *mRNA* expression of VEGF, α-SMA, and TGFβ-1 in rabbit are as follow: GAPDH: 5′-CAAGTTCAACGGCACAG-3′ and 5′-CCAGTAGACTCCACGACAT-3′, VEGF: 5′-AGTACCCTGATGAGATCGAGT-3′ and 5′-TTGTTGTGCTGTAGGAAGCT-3′, α-SMA: 5′-CACCACTCCTTCTACAATGA-3′ and 5′-GTCTCAAACATAATCTGGGTC-3′, TGF-β1: 5′-CAGAGGCTCAAGTTACAGCA-3′ and 5′-CACAACTCCAGTGACATCG-3′.

S1.5 Immunohistochemistry staining antibodies

We used the following antibodies to evaluate the protein expression in HS tissues in all groups by immunohistochemistry: anti-VEGF (Novus, NBP2-45235, 1:400), anti-α-SMA (Invitrogen, MA5-

11547, 1:800), anti-TGF-β1 (GeneTex, GTX21279, 1:500), anti-Collagen I (Novus, NB600-408, 1:100), anti-Collagen III (Novus, NBP1-0511, 1:400) primary antibodies, and an HRP-conjugated goat antirabbit secondary antibody (MaxVision, KIT-5020, 1:1).

S1.6 Wounding in rabbit ears

The animals were anesthetized with pentobarbital sodium (1 mg/kg). Then, four circular full-thickness wounds with a diameter of 10 mm were made on the ventral surface of each ear by removing the epidermis, dermis, and perichondrium to the bare cartilage.

In group A, immediately after modeling, the needle was inserted 2 mm from the upper, lower, left, and right edges of the wound, and about 0.1 mL of the drug was injected slowly under the skin using a 1mL syringe until the skin turned white, avoiding leakage of the drug.

In group B, a 1 mL syringe was used to enter the needle about 2 mm toward the center at the edge of each scar in the upper, lower, left, and right directions, and slowly injected about 0.1 mL of the drug underneath the skin until the scars become white, avoided leakage of drugs.

S1.6 Cell viability test of MPSS@ZIF-90 for normal cells.

BEND3 cells were seeded into 96-well plates at 5000 cells per well. Then, these cells were cultured in DMEM with 10% fetal bovine serum for 48 h. MPSS@ZIF-90 were added at designed concentrations and incubated for 1, 3, and 5 days. Relative cell viabilities of endothelial cells loaded with MPSS@ ZIF-90 (2.5 – 40 μg/mL) were evaluated by CCK-8 assay on day 1, 3, and 5.

Results and discussion

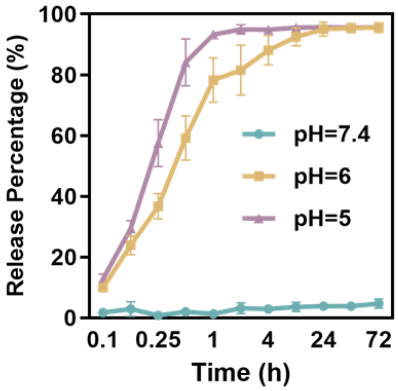

Figure S1. Release profile of MPSS@ZIF-90 at pH 5, pH 6, pH 7.4.

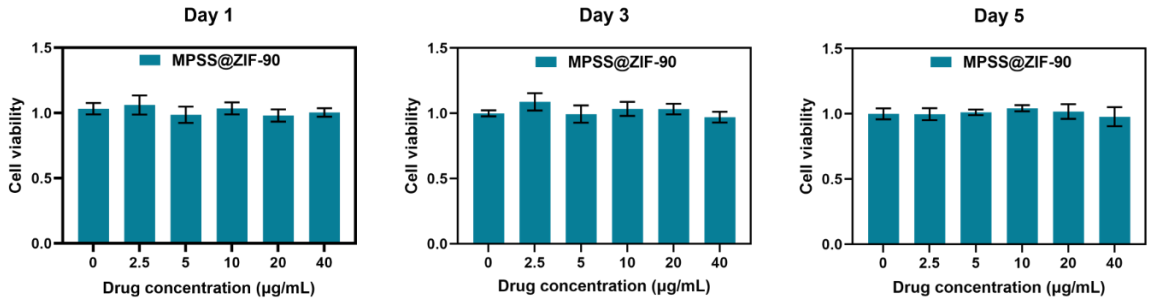

Figure S2. Cell viability of human vascular endothelial cells after being treated for 1, 3, and 5 days.

Equation S1:

SEI = H/H0

H represents the length between the highest point to the surfaces of cartilage in the scar, and H0 represents the length from the epithelium to the surface of the cartilage in adjacent normal skin. A ratio of 1 indicates no difference in height between the wound and unwounded skin.