Supporting Information

Vitamin metal-organic framework-laden microfibers from microfluidics for wound healing

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KEYWORDS: metal-organic framework (MOF); vitamin; microfiber; microfluidics; wound healing

Experimental Section:

Materials, cell lines, and animals

Sodium alginate, Nicotinic acid, and zinc acetate were purchased from Aladdin. Copper (II) acetate monohydrate was bought from Alfa Aesar. Calcium chloride (Anhydrous) was from Sigma-Aldrich. Fluorescent polystyrene nanoparticles (F8805 (excitation/emission: 365/415 nm) was from Invitrogen. Solutions were all filtered before pumped into glass micro-capillary devices. Water with a resistivity of 18.2 MΩ·cm^{-1} was acquired from a Millipore Milli-Q system. All other chemical reagents were of the best grade available and used as received.

NIH 3T3 cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Eagle's Minimum Essential Medium (EMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) under the condition of 37℃, 5% CO_2.

The 8-12 weeks, male Sprague-Dawley and BALBc mice were supplied by Jinling Hospital. Animals were treated in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA. All the animal care and experimental protocols were reviewed and approved by Animal Investigation Ethics Committee of Jinling Hospital.

Microfluidics

The capillary microfluidic devices were made up of coaxial compositions of round and square glass capillaries on glass slides. For the microfluidic device to fabricate copper- or zinc-MOF-laden microfibers, a round glass capillary tube (World Precision Instruments) with an orifice diameter of about 40-60 μm was coaxially inserted into a capillary with an orifice diameter of about 100 μm, which were then coaxially inserted into a tapered capillary with the orifice of about 300 μm. All of the tapered capillaries were then coaxially aligned in the collection capillary and acted as the core and shell injection channels in the microfluidics. The injection tubes and the collection capillary were then coaxially assembles into a square capillary with inner diameter 1.05 mm (AIT Glass). A transparent epoxy resin was used to seal the tubes where necessary. To fabricate the microfibers with multiple MOF cores, two or three sets of the injection channels were used to coaxially insert into the tapered capillary with a diameter of about 300μm. Then the integrated capillaries were used as an inner channel and coaxially assembled in the square capillary with a collection channel.

The outer phase was 2 wt% calcium chloride ($CaCl_2$) and the middle pre-gel aqueous phases were 2 wt% sodium alginate solutions with about 0.01% (v/v) fluorescent polystyrene nanoparticles. The inner phases were 0.1 mmol/mL nicotinic acid and 0.016, 0.05, 0.158, 0.5, 1.58 and 2 mM copper acetate or zincic acetate solutions. Since the concentration of nicotinic acid is much more than the metal ion precursor solutions, the yield of the MOFs is dependent on the concentration of different metal ion precursors.

Nicotinic acid release test of the microfibers

2wt% Na-Alg solution was pumped at the speed of 1mL/h, 2mL/h, and 3mL/h, respectively. The nicotinic acid solution was pumped at the speed of 0.2mL/h, and the different concentrations of the metal ion precursor solution were pumped at the speed of 0.12mL/h. All the fluids were flowed in the microfluidic device in 30min. The control group is made from 2 wt% Na-Alg solution and 2 wt% $CaCl_2$. The resultant MOF-laden microfibers were collected in the high concentrated vitamin solution and washed by ethyl alcohol, water and PBS solution twice, successively. Afterwards, the collected microfibers

were incubated into 1mL PBS (pH 7.4, 37°C) and shaken with a speed of 300rpm at 37°C. At predetermined intervals, 100μL of release media was taken out for content measurement and replenished with an equal volume of fresh media at 37℃. The release nicotinic acid was measured by UV-vis spectroscopy (Cary60, Agilent Technologies).

Cytotoxity tests of the vitamin MOF-laden microfibers

2 wt% Na-Alg solution was pumped at the speed of 1mL/h, 2mL/h, and 3mL/h, respectively. The nicotinic acid solution was pumped at the speed of 0.2mL/h, and the different concentrations of the metal ion precursor solution were pumped at the speed of 0.12mL/h. All the fluids were flowed in the microfluidic device in 30min. The resultant MOF-laden microfibers were collected and washed by ethyl alcohol, water and PBS solution twice, successively. The resultant microfibers were added into each well containing 1mL culture medium, and incubated for 48h. The NIH 3T3 fibroblasts were plated in 96-well cell culture dishes with 4000 cells/well (100 μL/well) for 24 h to allow attachment before the experiment. Then, the microfibers in the 96-well dish were removed, and the cells were rinsed with PBS, and the extract solutions were added into the wells with another fresh culture media, and the cells were incubated with the extract solutions for another 24h. The cells were then stained by calcein and propidium Iodide and the cell viability were measured by the confocal laser scanning microscope.

Antibacterial activity of the hybrid hydrogel

The Gram-negative bacteria *E. coli* were used to investigate the antibacterial activities of the hybrid hydrogels. Firstly, tested groups of microfibers were prepared as before and incubated in the 96-well plate with PBS buffer (pH 7.4) for 1h at 37°C. Subsequently, 100 μL of bacteria solution (10^4 CFU/mL) was added onto the surface of the hydrogels and incubated for 24 h at 37℃ after which the suspension was taken out. The suspension was further stained with a combination of dye solution (SYTO9, propidium iodide) in the dark for 15 min at 37°C. The stained bacteria were observed and measured by confocal laser scanning microscope.

Antioxidant activity of the hybrid hydrogel

The hydrogen peroxide and NIH 3T3 cells were used to build the cellular oxidative stress injury models. The control groups are normal NIH 3T3 cells and NIH 3T3 cells treated with 0.04% hydrogen peroxide; the experiment groups are NIH 3T3 cells treated with a mixed medium of 0.04% hydrogen peroxide and copper-MOF laden microfibers, zinc-MOF laden microfibers or combined MOFs-laden microfibers, respectively. The origin concentration of the metal ions was 0.158mM in each MOF containing group. The NIH 3T3 cells were plated in 96-well cell culture dishes with 4000 cells per well (100 μL) for 24 h to allow attachment before the experiment. After the microfibers were collected and washed by ethyl alcohol, water and PBS solution twice, successively, they together with hydrogen peroxide were induced into the cells, and co-cultured for 4 h. Then, the cell viability was measured by Counting Kit-8 test, and the control group was set as 100%.

Wound healing study of the hybrid hydrogel

A mouse full-thickness cutaneous infected wound model was used to evaluate the effect of vitamin MOF-laden microfibers on wound healing. First, a total of 24 healthy male Sprague-Dawley rats (180–250g, Jinling Hospital, Nanjing, China) were anesthetized and their backs were shaved. A rounded full-thickness cutaneous wound (1 cm × 1 cm) area was created on the back of each rat after which 200μL E. coli solution (1×10^8 CFU/mL) was introduced onto the wounds, and then divided into four groups randomly. The four groups were treated with Ca-Alg microfibers, zinc-MOF-laden microfibers, copper-MOF-laden microfibers and combined MOFs-laden microfibers respectively. The precursor solutions were sterilized by filtration using 200 nm syringe filters, and the saline was sterilized by autoclaving. Thereafter, the rats were individually housed in cages and allowed to heal for 9d. The wounds were observed on day 0, 3, 5, 7 and 9. The mice were sacrificed after 9d and granulation tissues over the wound bed were excised and then immersed in neutral formaldehyde for further histology and immunohistochemistry analysis.

Histology and Immunohistochemistry

The granulation tissue samples were removed from the neutral formaldehyde, followed by dehydration and embedded in paraffin. Serially sections, 5μm in thickness, were acquired by a microtome according to standard protocols, and were prepared for hematoxylin-eosin and immunohistochemical staining. Sections for immunohistochemistry were stained with CD31 and TNF-α.

Characterization

The microfluidic generation processes in the capillary microfluidic devices were observed in real-time with the help of microscope (AE2000, Motic) and was recorded by a fast camera (CCD, S-PRI F1, AOS Technologies AG). Bright-field images and fluorescence images were snapped by microscopy (OLYMPUS IX71) equipped with CCD cameras (Media Cybernetics Evolution MP 5.0 or Olympus DP30BW). Fluorescence photographs of cross-section of the microfibers with multiple vitamin MOF cores were snapped by a Laser Scanning Confocal Microscope (OLYMPUS, FV10i). The microstructures of the fabricated copper- and zinc-MOF-laden microfibers were characterized by a scanning electron microscope (SEM, HITACHI, S-3000N). The X-ray diffraction (XRD) was performed to characterize the crystal structure of the MOFs in the microfiber

core. The infrared spectra were collected with a Thermo Scientific Nicolet iS50 FTIR spectrometer. The porosity of the synthesized MOFs were characterized by Accelerated Surface Area Porosimetry System (ASAP2020M), and the specific surface area of the MOFs was only about 34.5 m^2/g.

Supporting Figures

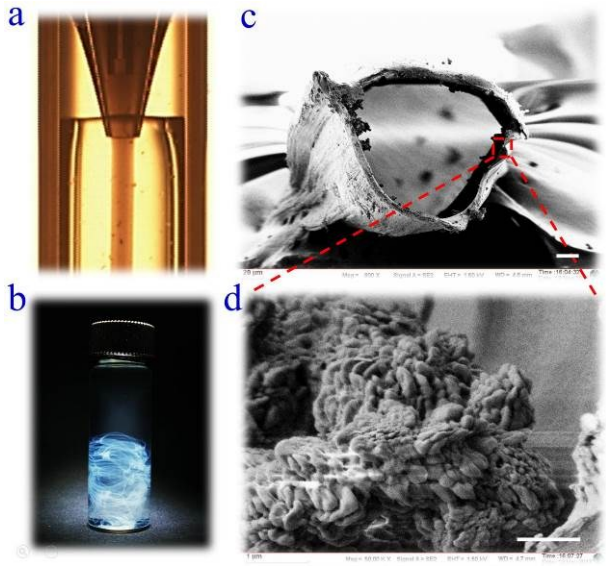

Fig. S1 (a) Real-time microscope image during the microfiber generation; (b) Digital image of the microfibers laden with copper-MOF in a vessel; (c, d) Scanning electron microscope (SEM) images of the (c) core-shell structured microfiber, and (d) the copper-MOF inside the fiber. The scale bars are (c) 20µm and (d) 1µm.

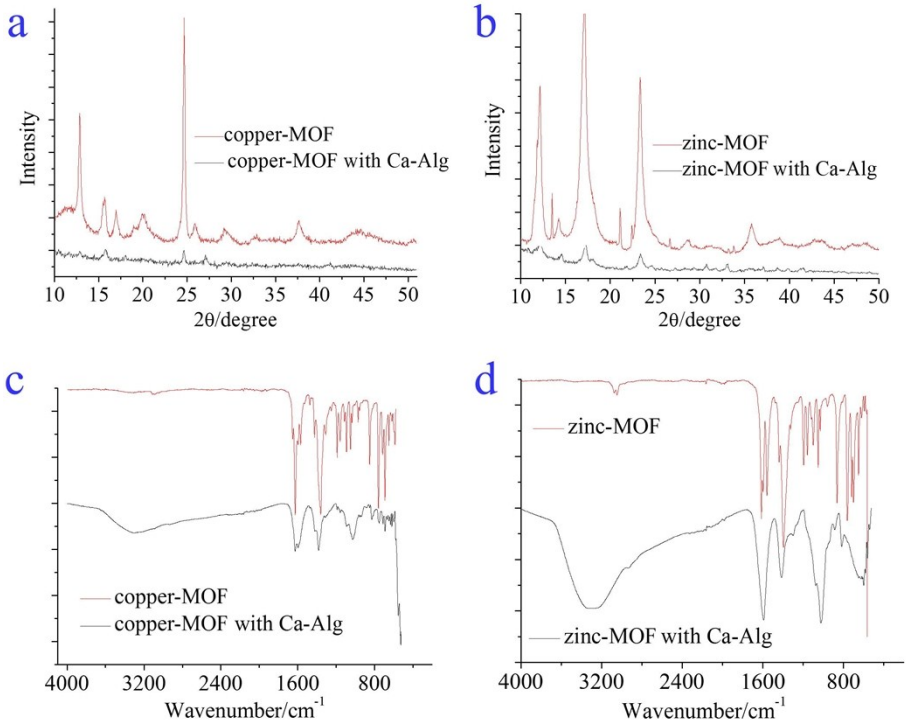

Fig. S2 (a, b) The X-ray diffraction (XRD) patterns of the (a) copper-, (b) zinc-MOFs and with Ca-Alg hydrogel; (c, d) The fourier transform infrared spectroscopy (FTIR) analysis of the (c) copper-, (d) zinc-MOF and with Ca-Alg hydrogel.

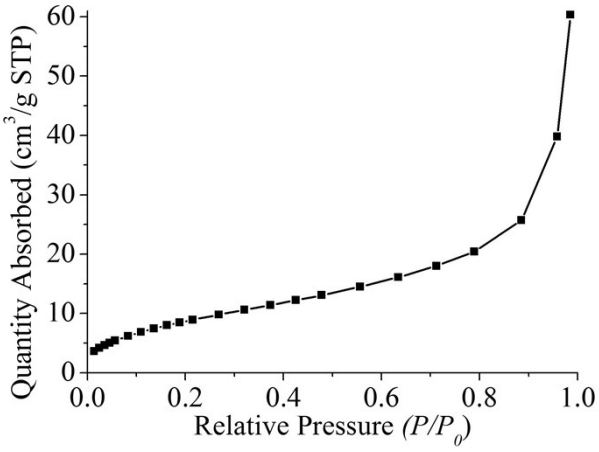

Fig. S3 The nitrogen absorption curve of the zinc-MOFs.

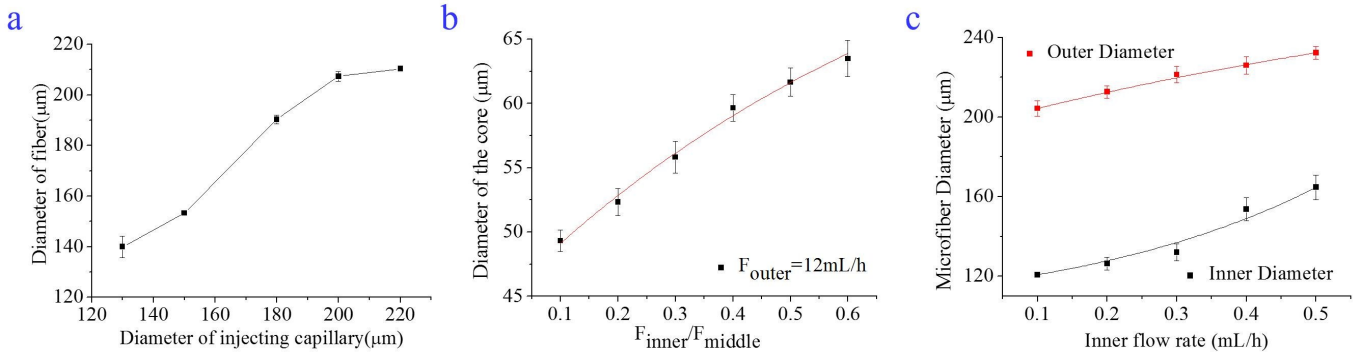

Fig. S4 (a) Relationship between the diameter of the microfiber and the diameter of injecting capillary; (b) Relationship between the diameter of the core structure of the microfiber and the ratio of inner flow rate to middle flow rate; (c) Relationships between the inner and outer diameters of the microfibers and the flow rates of the inner phase solution.

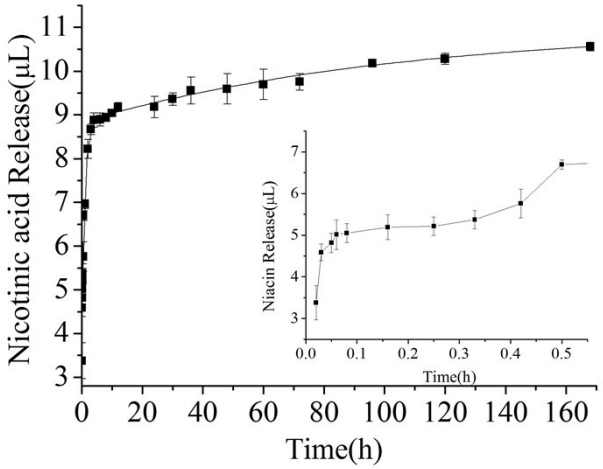

Fig. S5 The release condition for the copper-MOF in PBS solution and the downright curve is that within 30min.

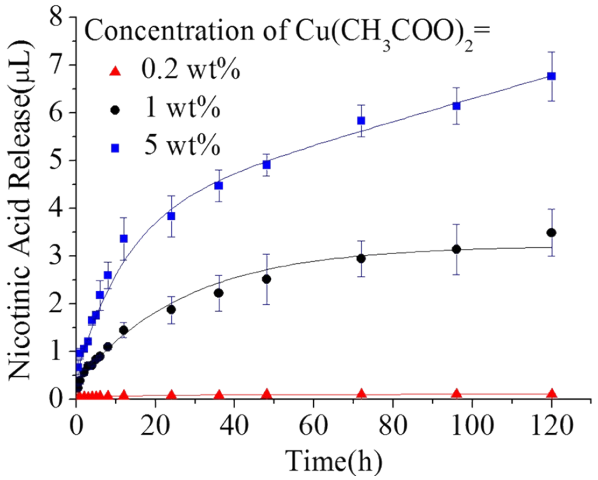

Fig. S6 The release of the MOF-laden microfibers with different concentration of precursors.

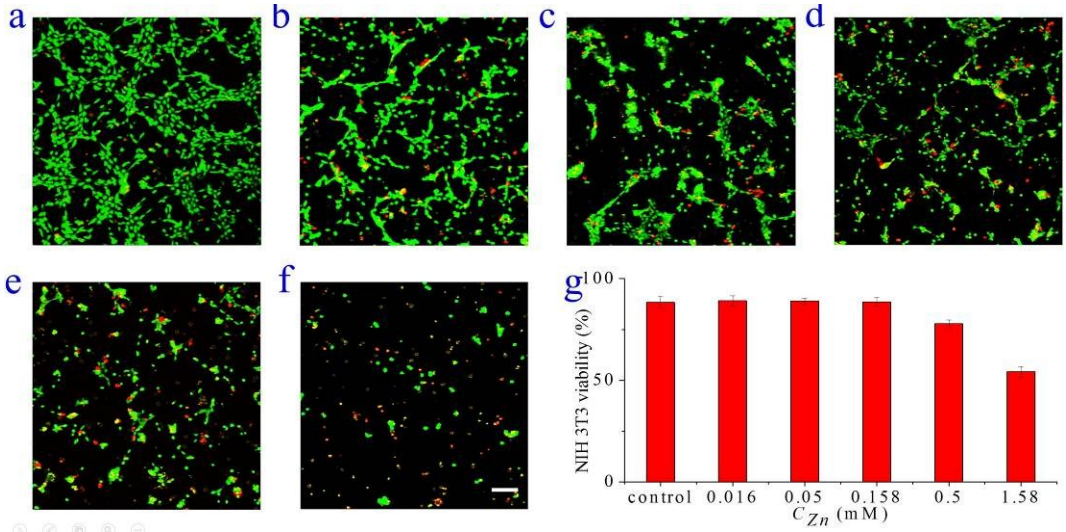

Fig. S7 (a-f) Confocal laser scanning images of the fluorescent stained cells on (a) Ca-Alg microfibers and (b-f) zinc-MOF-laden microfibers with the origin zinc concentrations of 0.016, 0.05, 0.158, 0.5, and 1.58mM, respectively. The scale bar is 50μm; (g) The statistical gram of the cell viability of the microfibers with different vitamin zinc-MOF-laden concentrations.

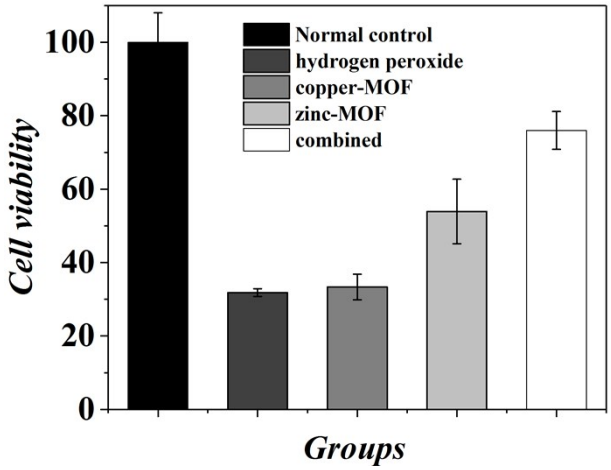

Fig. S8 Cell viabilities of NIH 3T3 cells. The groups are NIH 3T3 cells alone, cells treated with 0.04% hydrogen peroxide, cells treated with the mixture medium of 0.04% hydrogen peroxide and copper-MOF, zinc-MOF, or combined MOFs laden microfibers, respectively.

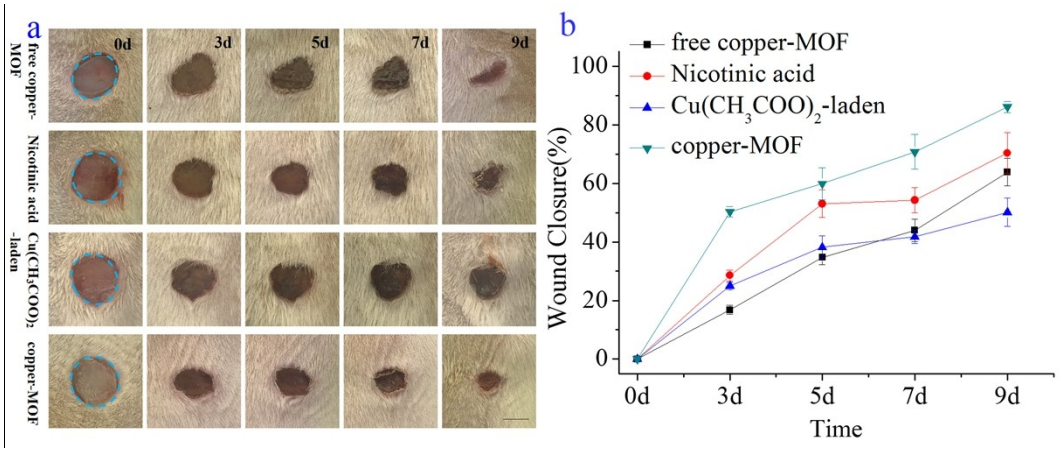

Fig. S9 (a) Representative photos of the skin wounds treated with free copper-MOF, nicotinic acid containing, $Cu(CH_3COO)_2$-laden and copper MOF-laden microfibers. The scale bar is 500μm; (b) The statistical gram of the wound closure.

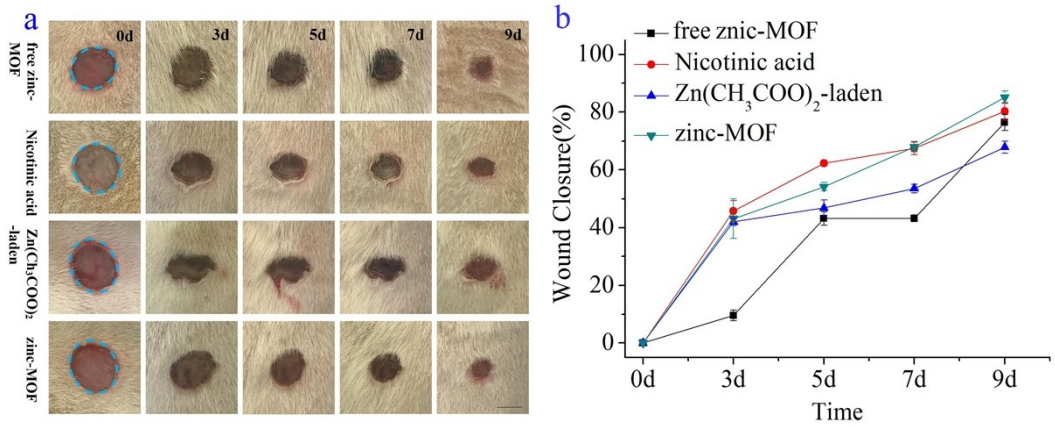

Fig. S10 (a) Representative photos of the skin wounds treated with free zinc-MOF, nicotinic acid containing, $Zn(CH_3COO)_2$-laden and zinc MOF-laden microfibers. The scale bar is 500μm; (b) The statistical gram of the wound closure.