

pH-responsive and hyaluronic acid-functionalized metal-organic frameworks for therapy of osteoarthritis

Feng Xiong^{1,2,3,4}, Zainen Qin^{1,2,5}, Haimin Chen^{1,2,5}, Qiumei Lan^{1,3,5}, Zetao Wang¹, Nihan Lan^{1,2,5,*}, Yuan Yang^{1,2,4,*}, Li Zheng^{1,2,*}, Jinmin Zhao^{1,2,3,6}, Dan Kai⁷

1 Guangxi Engineering Center in Biomedical Material for Tissue and Organ Regeneration, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, China

2 Guangxi Collaborative Innovation Center for Biomedicine, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, China

3 Department of Orthopaedics Trauma and Hand Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, China

4 Orthopaedics, Langdong Hospital of Guangxi Medical University, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, China

5 Life Sciences Institute, Guangxi Medical University, Nanning, 530021, China

6 Guangxi Key Laboratory of Regenerative Medicine, Life Sciences Institute, The First Affiliated Hospital of Guangxi Medical University, Nanning, 530021, China

7 Institute of Materials Research and Engineering (IMRE), A*STAR, 2 Fusionopolis Way, #08-03 Innovis, 138634, Singapore

* Co-Corresponding authors: Nihan Lan, Yuan Yang, Li Zheng

E-mail addresses: 1142963164@qq.com (Prof. Nihan Lan), yangy062@sina.com (Prof. Yuan Yang), and zhengli224@163.com, Fax: +86-07715306456 (Prof. Li Zheng)

¹ Feng Xiong, Zainen Qin, Haimin Chen contributed as first authors.

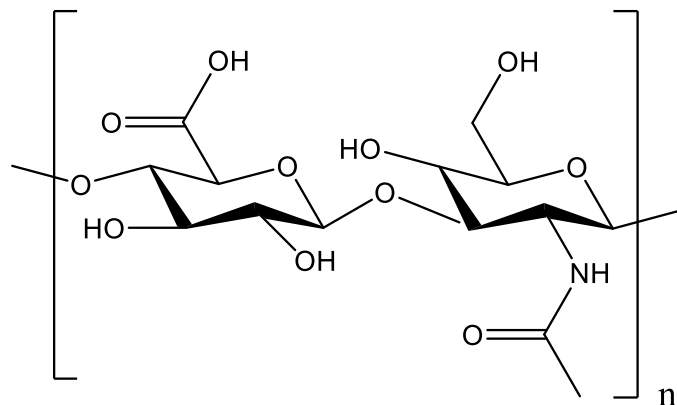


Figure S1. The chemical structures of HA (hyaluronic acid) [1, 2].

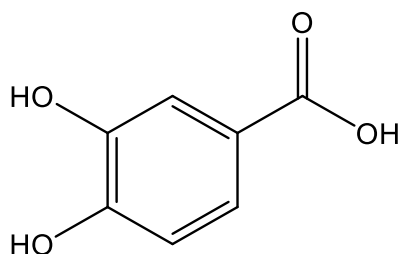


Figure S2. The chemical structures of PCA (protocatechuic acid) [3].

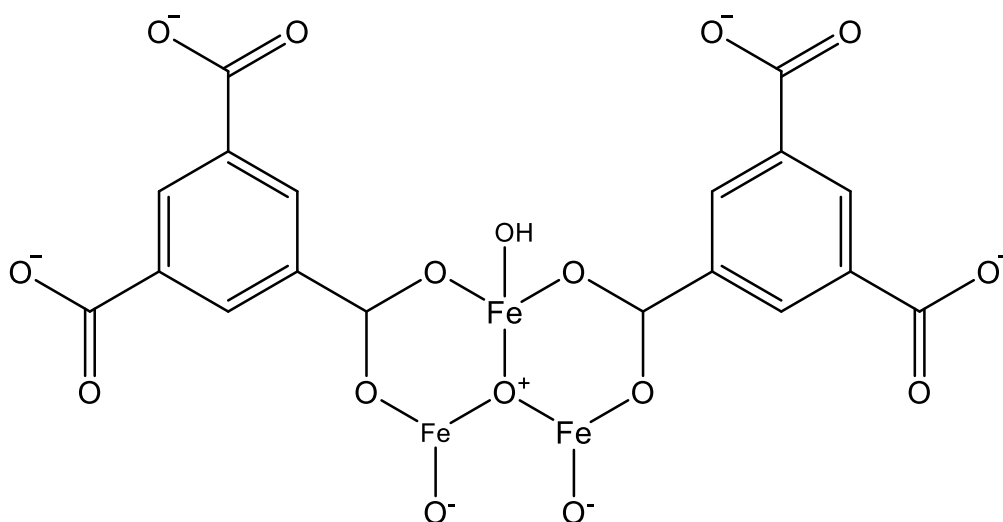


Figure S3. The chemical structures of MOF [4].

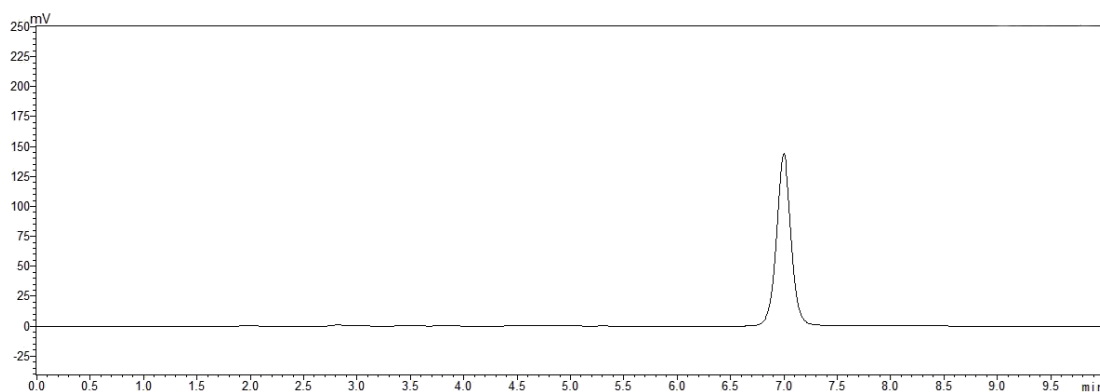


Figure S4. The typical chromatogram of PCA.

1. Quantify the amount of HA on MOF NPs

The MOF@HA NPs could be obtained by mixing MOF NPs with HA (Sigma-Aldrich, USA) at weight ratio of 2:1 for 24 h. The mixtures were washed with water for three times to remove the free HA, then MOF@HA NPs were obtained after lyophilization under vacuum. We quantified the amount of HA on MOF NPs and calculated the rate of HA on MOF NPs (HA, %) by the following formula:

$$\text{HA (\%)} = \frac{\text{weight of MOF@HA} - \text{weight of MOF feeding}}{\text{weight of MOF feeding}} \times 100\%$$

2. Quantitative measurement of released Fe³⁺ by the method of o-phenanthroline

[5]

The degradation of MOF was investigated by quantitative measurement of released Fe³⁺ at different pH (**Figure S5 & 6**). FeCl₃ (4 mM) was diluted to different concentration and reduced to Fe²⁺ by Vitamin C (10 mM, 1 mL) for 5 min, then the mixture was reacted with o-phenanthroline (0.1%, 1 mL) and the red complex was produced, which absorbance is at 510 nm. The volume of the reaction system was 5 mL. The absorption spectra of different samples were obtained using an UV-vis spectrometer. The absorbance intensity at 510 nm was correlated with the Fe³⁺ in concentration. The

obtain standard curve is $y=0.00548x+0.04226$ (y: absorbance value at 510 nm; x: concentration of Fe^{3+} , $R^2=0.9998$) (Fig. S5).

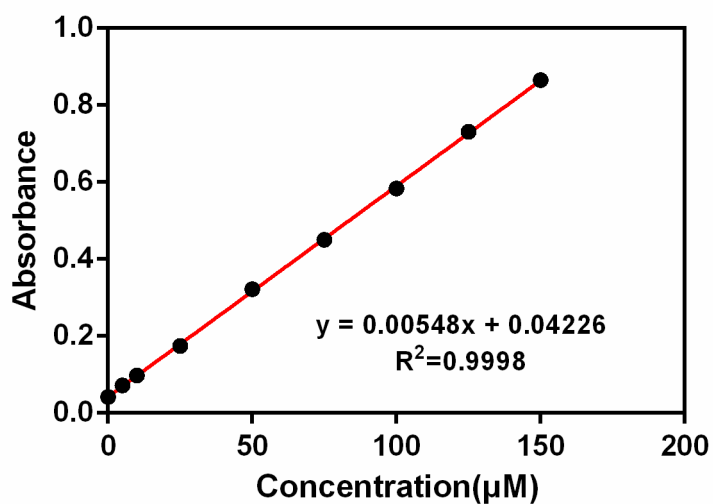


Figure S5. The quantification of Fe^{3+} by using o-phenanthroline. The absorption spectra of inset: The standard curve for absorbance value at 510 nm. $y=0.00548x+0.04226$, $R^2=0.9998$.

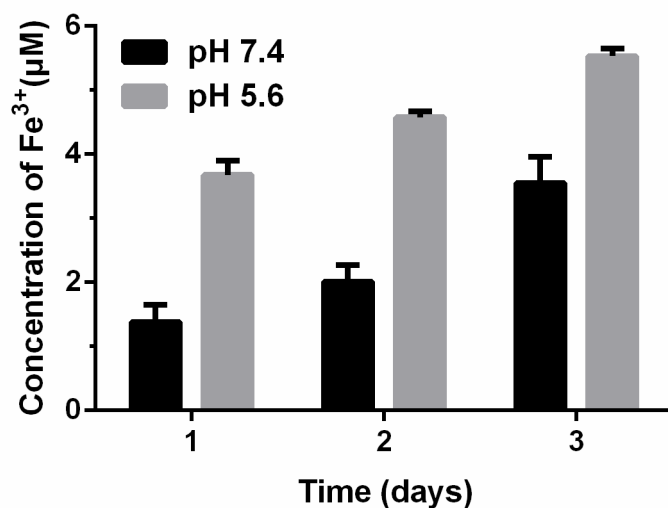


Figure S6. Degradation study of MOF at different pH. The quantitative analysis of the Fe^{3+} released from MOF in various pH environment. The data represents the mean \pm SD (n=3).

3. ROS production from MOF in chondrocytes

Reactive Oxygen Species Assay Kit (Beyotime Biotechnology, China) was used to detect the ROS generation of intracellular induced by MOF [6, 7].

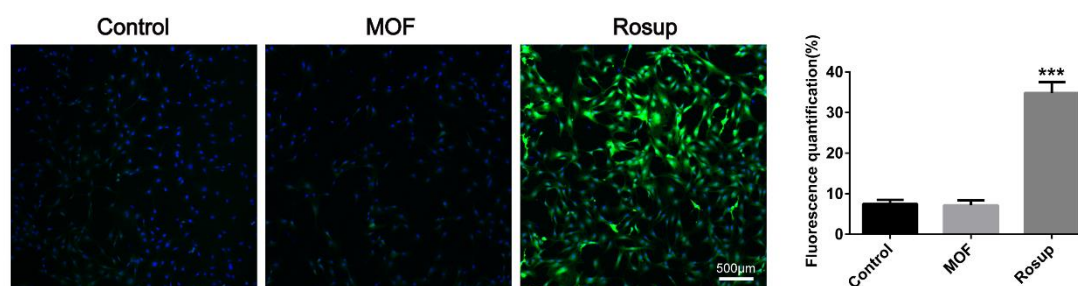


Figure. S7 ROS production induced by MOF after incubation in chondrocytes for 24h. Data was presented as the mean \pm SD (n=3). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

- [1] Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. *Advanced materials*. 2011;23(12):H41-56.
- [2] Highley CB, Prestwich GD, Burdick JA. Recent advances in hyaluronic acid hydrogels for biomedical applications. *Current opinion in biotechnology*. 2016;40:35-40.
- [3] Krzysztoforska K, Mirowska-Guzel D, Widy-Tyszkiewicz E. Pharmacological effects of protocatechuic acid and its therapeutic potential in neurodegenerative diseases: Review on the basis of in vitro and in vivo studies in rodents and humans. *Nutritional neuroscience*. 2019;22(2):72-82.
- [4] Tahereh AV, Reza NJM, Leila P. (Fe)MIL-100-Met@alginate: a hybrid polymer-MOF for enhancement of metformin's bioavailability and pH-controlled release. *New Journal of Chemistry*. 2018:10.1039.
- [5] Wang D, Zhou J, Chen R, Shi R, Xia G, Zhou S, et al. Magnetically guided delivery of DHA and Fe ions for enhanced cancer therapy based on pH-responsive degradation of DHA-loaded Fe₃O₄@C@MIL-100(Fe) nanoparticles. *Biomaterials*. 2016;107:88-101.
- [6] Sun L, Chen L, Bai L, Xia Y, Yang X, Jiang W, et al. Reactive oxygen species mediates 50-Hz magnetic field-induced EGF receptor clustering via acid sphingomyelinase activation. *International journal of radiation biology*. 2018;94:678-684.
- [7] Deng S, Dai G, Chen S, Nie Z, Zhou J, Fang H, et al. Dexamethasone induces osteoblast apoptosis through ROS-PI3K/AKT/GSK3 β signaling pathway. *Biomed Pharmacother*. 2019;110:602-608.