Supporting Information

pH and Enzyme Dual-Responsive Releasing of H2S for Disc Degeneration Therapy

Zengming Zheng, Anqi Chen, Huacheng He, Yu Chen, Jian Chen, Abdullkhaleg Ali Albashari, Jiawei Li, Jiayu Yin, Zili He, Qingqing Wang, Jiang Wu, Qian Wang, Jianming Kang, Ming Xian, Xiangyang Wang and Jian Xiao

a Department of Orthopaedic Surgery, The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, 325035, P.R. China

b School of Pharmaceutical Sciences, Key Laboratory of Biotechnology and Pharmaceutical Engineering, Wenzhou Medical University, Wenzhou, Zhejiang, 325035, P.R. China

c College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou, Zhejiang, 325035, P.R. China

d Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208, United States

e Department of Chemistry, Washington State University, Pullman, Washington 99164, United States

Corresponding authors: Huacheng He, hehc@wzu.edu.cn; Xiangyang Wang, xiangyangwang2016@163.com; Jian Xiao, xfxj2000@126.com
Experimental Section

S1. Degradability of collagen hydrogel

Collagen extract from rat tails were made into hydrogels with cubic morphology. The hydrogels were immersed into PBS (pH 7.4) with or without MMP9 (50 μg/mL). Then the hydrogels were put into a 37 °C incubator for 12 h. After 12 h, the hydrogels were taken out of the incubator for imaging.

S2. Immunohistochemical examination

After 16 weeks, all discs were embedded in paraffin and cut into sections (5 mm). The sections were deparaffinized in xylene, and rehydrated by ethanol washing. Next, 3% (v/v) hydrogen peroxide was used to block endogenous peroxidase activity for 10 min, and 5% bovine serum albumin was used to block nonspecific binding sites for 30 min at room temperature. The sections were then incubated with the primary antibody (anti-Cystathionase) overnight at 4°C. After primary antibody incubation, the sections were incubated with an appropriate HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Dallas, TX, USA) and counterstained with hematoxylin. Images were captured using a light microscope.
Figure S1. The study of degradability of collagen hydrogel at the presence of MMP. Collagen extracted from rat tails was made into cubic shape hydrogels and incubated in PBS (pH 7.4) with or without MMP9 (50 μg/mL) at 37 °C for 12 h. As clearly presented in the figure, the hydrogel treated with MMP9 becomes thinner and completely loses its cubic morphology, while the collagen hydrogel without MMP9 treatment still maintains its cubic shape.

Figure S2. Immunohistochemical staining of CSE (Top) and the bar diagram of the relative expression of CES after treatments of Col and Col-JK1. Scale bar is 200 μm. Significant differences is indicated as ***P<0.001, n=6. As shown in the figure, Col group exhibits
extremely low expression of CSE. To the contrary, Col-JK1 group shows significantly high expression of CSE, implying the release of H$_2$S from Col-JK1 within the NP tissues of degenerated discs.$^1$

References