Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary information

S-doped Carbonized Polymer Dots Inhibit Early Myocardial Fibrosis by Regulating Mitochondrial Function

Yiran Wanga#, Mingxi Yangbc#, Jiayi Zhangb, Jingyan Renb, Ning Liua and Bin Liua*, Laijin Lub*, and Bai Yangc*

# Yiran Wang and Mingxi Yang contributed equally to this work.

a Department of Cardiology, The Second Hospital of Jilin University, Changchun 130021, P.R. China

b Department of Hand Surgery, The First Hospital of Jilin University, Changchun 130021, P.R. China

c State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, P.R. China

Experimental section

Cytotoxicity and proliferation test

H9c2 cells were inoculated in a 96-well plate (5×10^3 cells/well) in a final volume of 100 μl, and stimulated with S-CPDs, L-cysteine, H_2O_2 and ISO. After stimulation, H9c2 cells were washed with PBS, incubated with 10 μL/well MTS (Promega, g358c, USA) at 37 ℃ for 1 h, and the survival rate of H9c2 cells was detected using an enzyme labeling instrument at 490 nm.

DCFH-DA fluorescence

ROS production was detected using a DCFH-DA fluorescent probe (Best bio, China). H9c2 cells were seeded in a 6-well plate (10^5 cells/well). After 24 h, the cells were stimulated with H_2O_2, S-CPDs+H_2O_2 and L-cysteine +H_2O_2. Next, the cells were washed with PBS and incubated with the DCFH-DA fluorescent probe (1:1000) diluted with serum-free DMEM at 37 ℃ for 20 min. The green fluorescence intensity of cells was detected. The cellular ROS level was proportional to the fluorescence intensity.

Animal modeling and grouping

Male Sprague Dawley (SD) rats aged 3-4 weeks (200 ± 20g) (Beijing Wei Tong Li Hua experimental animal Co. Ltd.) were kept in a room with a 12 h light/dark cycle at a constant temperature of 23 ℃, and provided commercial food and drinking water. The animal experiment was completed in the laboratory of the first hospital of Jilin University. Rats were randomly divided into the sham operation (Sham), ISO, S-CPDs + ISO, L-cysteine + ISO, Betaloc + ISO groups. Rats in the ISO group were subcutaneously injected with 200 μl ISO (5 mg/kg. day) for 15 days to establish the early MF model. Rats in the S-CPDs + ISO group were treated with both ISO (5 mg/kg. day) and S-CPDs (0.5 mg/kg. day) for 15 days. Rats in the L-cysteine + ISO group were treated with both ISO (5 mg/kg. day) and L-cysteine (0.5 mg/kg. day) for 15 days. Rats in the Betaloc + ISO group were treated with both ISO (5 mg/kg. day) and Betaloc (0.5 mg/kg. day) for 15 days. The rat weight was measured before treatment and on the 7th and 15th day during treatment. At the end of the experiment, HE staining and Masson's trichrome staining were observed the infiltration of inflammatory cells in Sham, ISO, ISO + L-cysteine, ISO +S-CPDs, ISO + Betaloc groups.

Immunohistochemistry

After 15 days of MF treatment, the rats were euthanized and the heart tissue was collected. The tissue was fixed with 4% formalin, dehydrated, paraffin embedded and sectioned (5 μM). Then, hematoxylin eosin (HE) staining and Masson's trichrome staining were performed according to the manufacturer's instructions. At the same time, the expression of connexin 43 protein in cells and mitochondria in tissues were detected using specific antibodies against Cx43 (Cell Signaling Technology，#3512，1：100) and Tom20 (Proteintech，11802-1-AP，1：100).

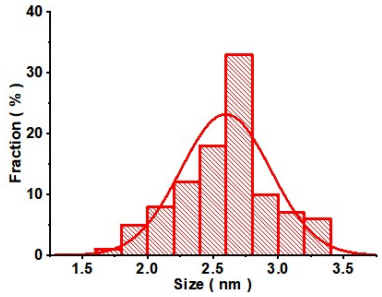

Figure. S1 The size distribution of S-CPDs.

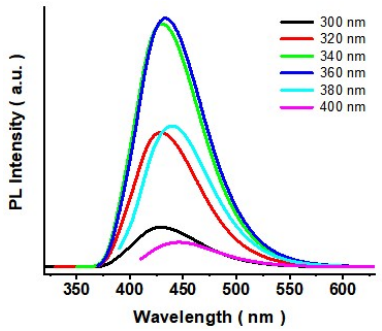

Figure. S2 The PL spectrum of S-CPDs.

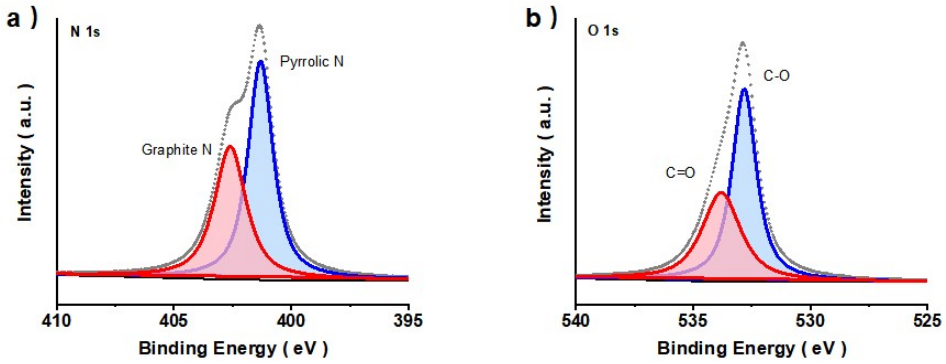

Figure. S3 The a) N 1s and b) O 1s deconvoluted XPS spectra.

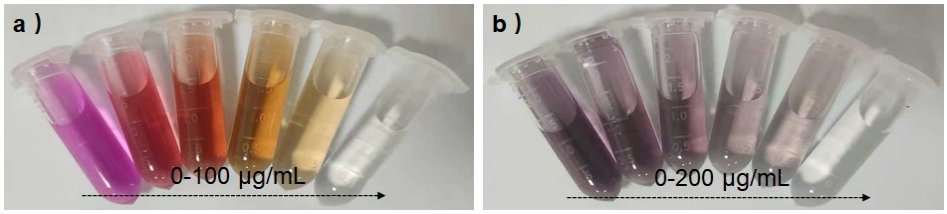

Figure. S4 The photographs of a) $KMnO_4$ reducing and b) Fenton reaction inhibiting experiments.

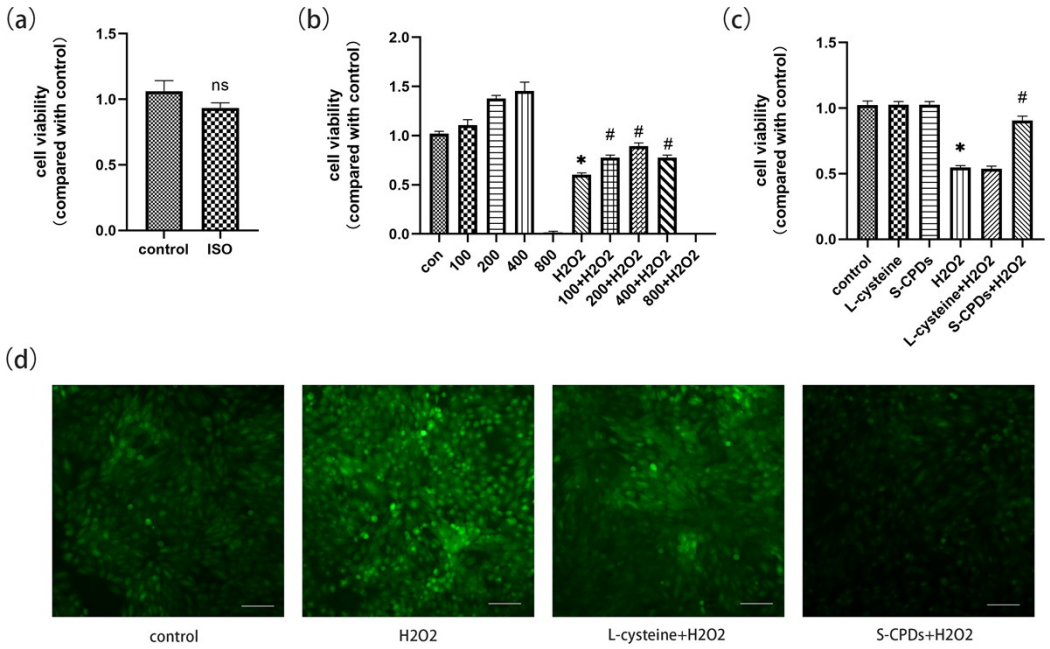

Figure. S5 a) The effect of ISO on cell mortality. b) Under H_2O_2 stimulation, MTS assay kit was used to detect the effect of different gradient S-CPDs on the H9c2 viability. c) The effect of L-cysteine and S-CPDs on cell survival under H_2O_2 stimulation. d) Under H_2O_2 stimulation, DCFH-DA kit was used to detect the effect of L-cysteine and S-CPDs on the level of ROS, Scale bar 160μm. *$p^{*} < 0.05$ vs Control ，$p^{\#} < 0.05$ vs ISO*

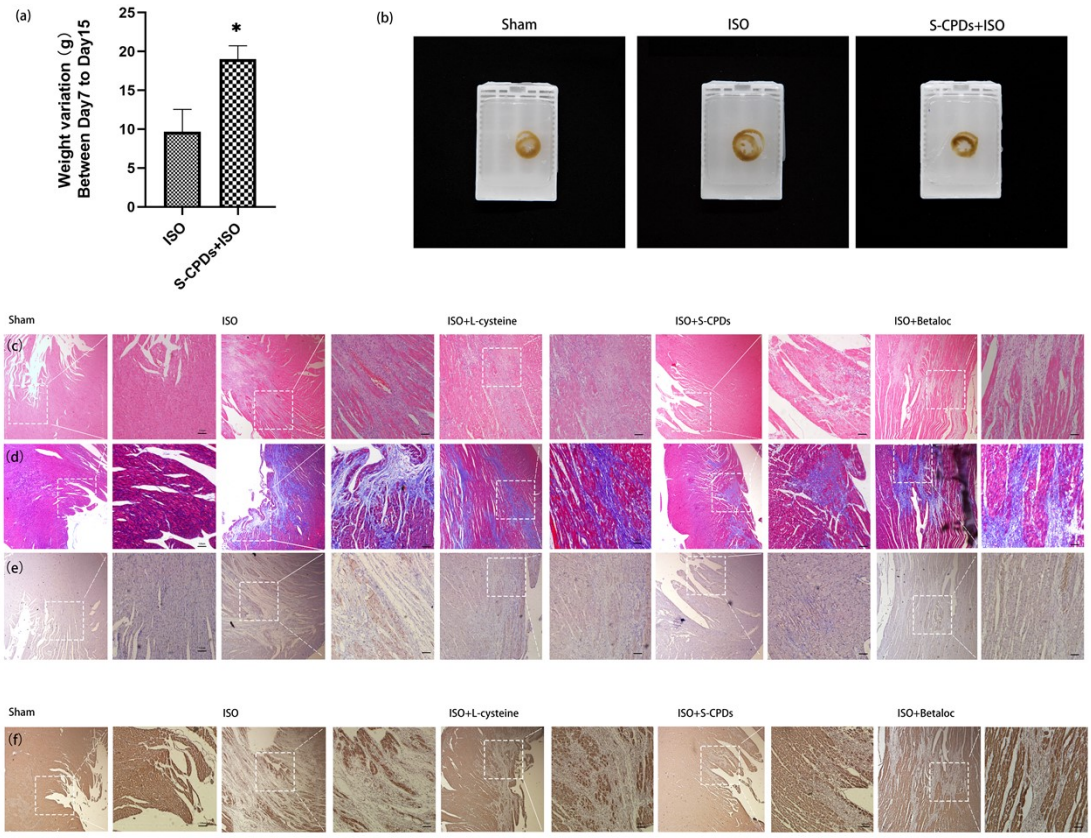

Figure. S6 a) SD rats weight variation in ISO, S-CPDs + ISO group were measured on the 15th and 7th day and weight variation was calculated. b) Paraffin-embedded heart tissue sections of Sham, ISO, S-CPDs +ISO groups. c-d) HE staining and Masson staining was used to observe the infiltration of inflammatory cells in Sham, ISO, ISO + L-cysteine, ISO +S-CPDs, ISO + Betaloc groups. Scale bar 120μm. e-f) Immunohistochemistry detected cardiac tissues' Cx43 and TOM20 expression Sham, ISO, ISO + L-cysteine, ISO +S-CPDs, ISO + Betaloc groups. Scale bar 120μm. $p^{*} < 0.05$ *vs Sham* , $p^{\#} < 0.05$ *vs ISO.*