Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Single-Atom-Anchored Microsweepers for *H. pylori* Inhibition through Dynamically Navigated Reciprocating Locomotion

Xinqi Cai,[a] Zhiyang Li,[a] Wen-jing Zhou,[a] Hui Deng,[a] Xiaoxu Cao,[a] Jieqiong Xu,[a] Zhiwei Yin,[a] Shen Wang,[a] Xin Xia,[a] Chao Ma,[b] Long Chen,[c] Ding Ding,*[d] Weihong Tan [a,d,e] and Zhuo Chen*[a]

[a]Molecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Life Sciences, Aptamer Engineering Center of Hunan Province, Hunan University, Changsha, Hunan, 410082, China

[b]College of Materials Science and Engineering, Hunan University, Changsha, Hunan, 410082, China

[c]Faculty of Science and Technology, University of Macau, Taipa, 999078, Macau

[d]Institute of Molecular Medicine (IMM), Renji Hospital, School of Medicine, College of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai, 200127, China

[e]The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Hangzhou Institute of Medicine (HIM), Chinese Academy of Sciences, Hangzhou, Zhejiang, 310022, China

Supporting Information

Table of content

Materials. Gastric pepsin, C_{18}-PEG, Mucin type II, Fumed silica, and TMB were purchased from Sigma-Aldrich. Chitosan was purchased from Macklin. Hemin was purchased from Aladdin. Ltd. RPMI 1640 medium was obtained from Invitrogen. CCK-8 was obtained from New Cell & Molecular Biotech (Jiangsu, China). Ultrapure water was obtained from Milli-Q Integral System, and water was double distilled at a resistivity of ~18.2 M/cm. *Helicobacter pylori* (ATCC 43504) was purchased from American type culture collection (ATCC). The GES-1 cell line was obtained from iCell Bioscience Inc (Shanghai, China). BALB/c mice (4-6 weeks old) were obtained from the Hunan SLRC Laboratory Animal Co., Ltd. (Changsha, China) and used under protocols approved by the Institutional Animal Care and Use Committee of Hunan University. All other chemical reagents were analytical grade and used without purification. Other chemicals are all commercially available.

Synthesis and characterization of MGNs and HMGNs. MGNs was prepared by the chemical vapor deposition (CVD) method as previously reported. Briefly, $Fe(NO)_3 \cdot 9H_2O$ (0.145 g) and $Co(NO)_3 \cdot 6H_2O$ (0.105 g) were loaded on fumed silica by ultrasound in methanol solution and dried to obtained the complex of cobalt salt, iron salt and silica. Then, the complex was used to obtain the MGNs loaded fumed silica through CVD with 5-min-methane for graphene growth at 800 °C. After that, the silica was etched by HF, and the MGNs were collected with the assistance of a magnet. Finally, C_{18}-PEG was used to modify MGNs to improve their water solubility.

HMGNs was synthesized by the secondary growth of the MGNs mixed Hemin in CVD at 600 °C. Hemin (an iron-containing porphyrin) was loaded onto the surface of MGNs through π-π stacking interaction as the single Fe atom precursor. Briefly, the MGNs loaded fumed silica was mix with Hemin (mass ratio 1:0.01) through ultrasound in methanol solution. After that, the compound was used for CVD with 25 min in hydrogen at 600 °C to obtain the HMGNs loaded fumed silica. Then, the HMGNs loaded fumed silica was etched by HF to dissolve the silicon. Finally, C_{18}-PEG was used to modify HMGNs to improve their water solubility. TEM imaging was tested by Themis Z (Thermo Scientific). UV–Vis spectrum was obtained by Shimadzu spectrophotometer (UV-2450).

Synthesis and characterization of FCSMGNs and FCSHMGNs. The microsweepers

were prepared using an electrostatic spraying method. Briefly, the mixture of fucoidan (1 %), chitosan (4 %) and magnetic graphitic MGNs or HMGNs (10 mg/mL) was electrostatically sprayed in a 10 kV-electrostatic field and cross linked by glutaraldehyde (0.75 %) to obtain the FCSMGNs or FCSHMGNs. The SEM imaging was tested by field emission electron microscopy (Tecnai G2F20S-TWIN). FT-IR spectra were collected using a Thermo Scientific Nicolet iS5. Field-dependent magnetization hysteresis loop was tested by LakeShore 7404.

***H. pylori* culture.** *H. pylori* was cultured using Columbia agar plate with 6.5% sterile Defibrinated Sheep Blood and multiple antibiotics (10 μg/mL vancomycin, 5 μg/mL trimethoprim lactate, 5 μg/mL cefsulodin sodium, and 5 μg/mL amphotericin B) at 37 °C, with the microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) for 3-5 days. The obtained *H. pylori* was transferred to DPBS for using.

***H. pylori* capture ability of the microsweepers.** The *H. pylori* capture capability of the FCSMGNs in simulated gastric fluid (SGF) was examined using fluorescent microscopy. *H. pylori* were labeled by Cy5 and co-incubation with FCSMGNs and CSMGNs (microcapsule consist of chitosan and MGNs but not fucoidan) in SGF under a 200-rpm shaking for 30 min, after that the capsules were removed using the external applied magnetic field.

The capability of the FCSMGNs capturing *H. pylori* in gastric mucus was examined using plate colony counting method. Briefly, *H. pylori* in mucus were co-incubated with the FCSMGNs and CSMGNs for 30 minutes (200 rpm shaking), after the microcapsules were removed, the residual mucus were collected for plate colony counting to investigate the number of uncaptured bacteria.

RPM-driven ability of the microsweepers. To evaluate the FSCMGNs' large motion function as sweepers, which would enable the adequate interaction between the FSCMGNs and bacteria, the FSCMGNs were added to the 24 well-plate or dish and observed the motion trajectory of the FCSMGNs with or without the application of the RPM.

RPM-driven enhanced *H. pylori* capture ability of the microsweepers in SGF. *H. pylori* was labeled by Cy5 through co-incubation for 30 min, and the excess dye was removed by centrifugation. Labeled *H. pylori* solution (DPBS, OD=0.2) and FCSMGNs (5mg) were added into SGF in 24-well plate and the 24-well plate was placed in the magnetic stirring apparatus (100 rpm) for 30 min. After that, the FCSMGNs was removed by a magnet, and the supernatant was observed through a Nikon TI-E+ A1 SI confocal laser scanning microscope (Japan) to assess the bacteria capture ability of the microsweepers.

RPM-driven enhanced *H. pylori* capture of microsweepers in gastric mucus. *H. pylori* solution (OD=1, 200 μL) and FCSMGNs (5mg) were added into gastric mucus in 24-well plate and the 24-well plate was placed in the magnetic stirring apparatus (100 rpm) for 30 min. After that, the FCSMGNs was removed by a magnet, and the supernatant was added into the Columbia agar plate for *H. pylori* culture. The number of the bacterial colonies was obtained by the Fiji software to evaluated the *H.pylori*-capture ability.

The oxidase-like property of HMGNs. TMB were incubated with the HMGNs in pH 4.5 buffer solution for 5 min and the absorbance was obtained by Shimadzu spectrophotometer (UV-2450). The HMGNs were incubated with TMB in different pH conditions to obtain the optimal pH conditions. For the EPR assay, DMPO was added to solution of HMGNs (pH 4.5) to capture ROS. EPR measurements were measured using a Bruker A300.

The stability and cytotoxicity of FCSHMGNs. The FCSHMGNs was added into water and SGF to observed the morphology stability of the FCSHMGNs, after 24-hour incubation, the absorbance of the supernatant was measured to confirm any particle leakage. To assess the catalytic property stability of the FCSHMGNs, the FCSHMGNs were incubated with TMB (pH 4.5) for 10 min, after that the supernatant was collected for UV-Vis assay and a new TMB solution was added to continue the reaction for six cycles.

Human gastric epithelial cells (GES-1) were seeded with a density of 5×10^3 cells per well

in the 96-well plate for 24 h at 37 °C. Then the microsweepers (0, 5, 8, 10 mg/mL) were added and incubated with the cells for 24 h. Afterwards, the cell viability was measured by Bio-Tek Multi-Mode Microplate Reader (Synergy 2, Winooski, VT) using the cell counting kit-8 (CCK-8).

RPM-driven enhanced *H. pylori* killing ability of FCSHMGNs in SGF. *H. pylori* solution (DPBS, OD=2) and catalytic FCSHMGNs (5 mg) were added into SGF in 24-well plate and the placed in the magnetic stirring apparatus (100 rpm) for 30 min. After that, the FCSHMGNs was removed by a magnet, and the bacteria was stained with AO and PI. Then, the live/dead *H. pylori* was observed through a Nikon TI-E+ A1 SI confocal laser scanning microscope (Japan) to assess the bacteria killing ability.

RPM-driven enhanced *H. pylori* killing ability in gastric mucus. *H. pylori* solution (OD=1, 300 μL) and FCSHMGNs (5 mg) were added into gastric mucus in 24-well plate and the 24-well plate was placed in the magnetic stirring apparatus (100 rpm) for 30 min. After that, the FCSHMGNs was removed by a magnet, and the supernatant was added into the Columbia agar plate for *H. pylori* culture and obtained the number of the bacterial colonies to assess the *H. pylori* killing ability.

Live subject statement

All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and used under protocols approved by the Institutional Animal Care and Use Committee of Hunan University.

RPM-driven enhanced *H. pylori* capture and killing in isolated mice stomachs. BALB/c female mice (4-6 weeks old) were obtained from the Hunan SLRC Laboratory Animal Co., Ltd. (Changsha, China) and used under protocols approved by the Institutional Animal Care and Use Committee of Hunan University. To collect the stomach, mouse was sacrificed, and the stomach was removed from the mouse body. *H. pylori* were injected into freshly dissected mouse stomachs, incubated for 30 minutes, after that the sweepers

(5 mg/mL) were added into the stomachs and placed on a magnetic stirrer for 30 minutes. And then, the gastric tissue was homogenized for plate colony incubation and counting.

Supplementary Figures

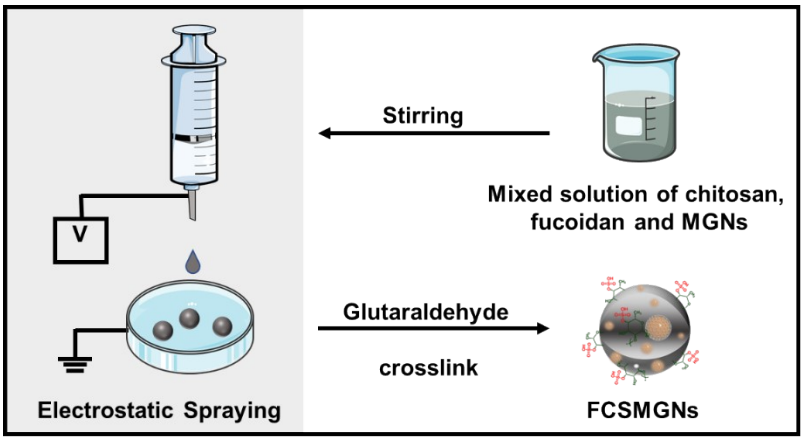

Figure S1. The scheme of FCSMGNs preparation using electrostatic spraying method. Briefly, the mixture of fucoidan, chitosan and magnetic graphitic MGNs was electrostatically sprayed in a 10 kV-electrostatic field and cross-linked by glutaraldehyde to obtain FCSMGNs.

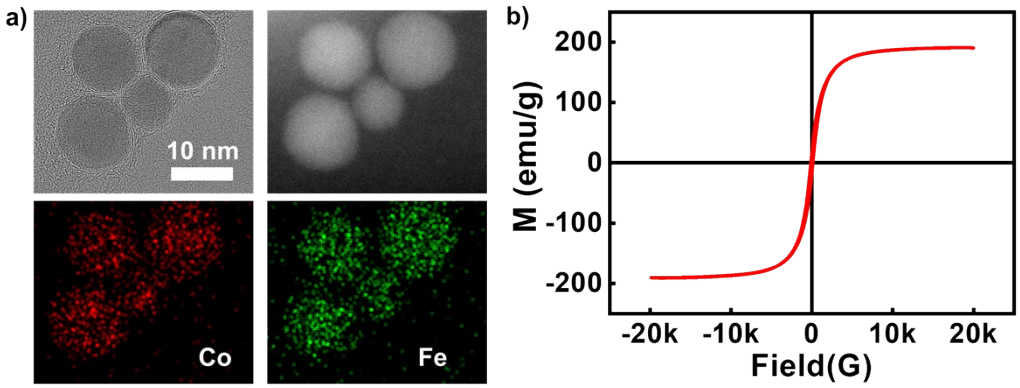

Figure S2. Characterization of MGNs. (a) TEM image and corresponding EDS mapping of MGNs. (b) Field-dependent magnetization hysteresis loop of the MGNs at room temperature.

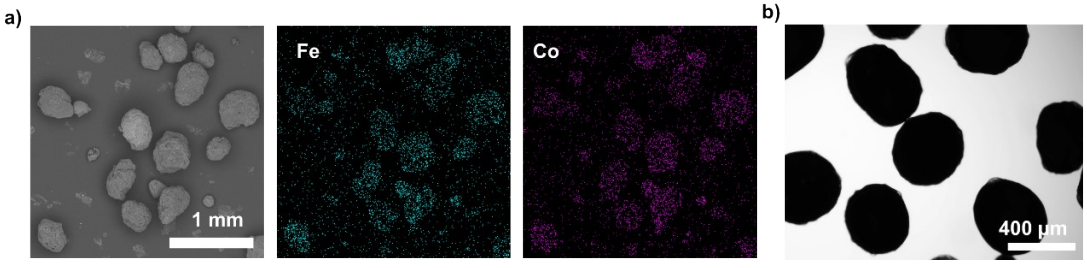

Figure S3. Characterization of FCSMGNs. (a) SEM image and corresponding EDS mapping of FCSMGNs. (b) Microscopy imaging of FCSMGNs.

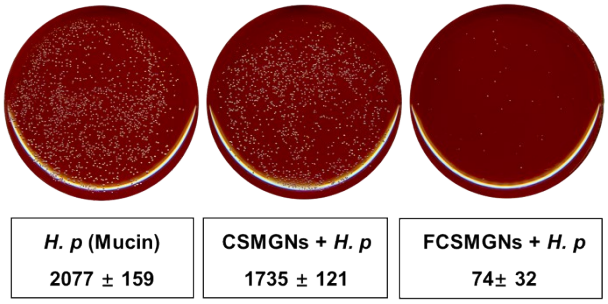

Figure S4. Photographs of *H. pylori* colonies treated with CSMGNs and FCSMGNs.

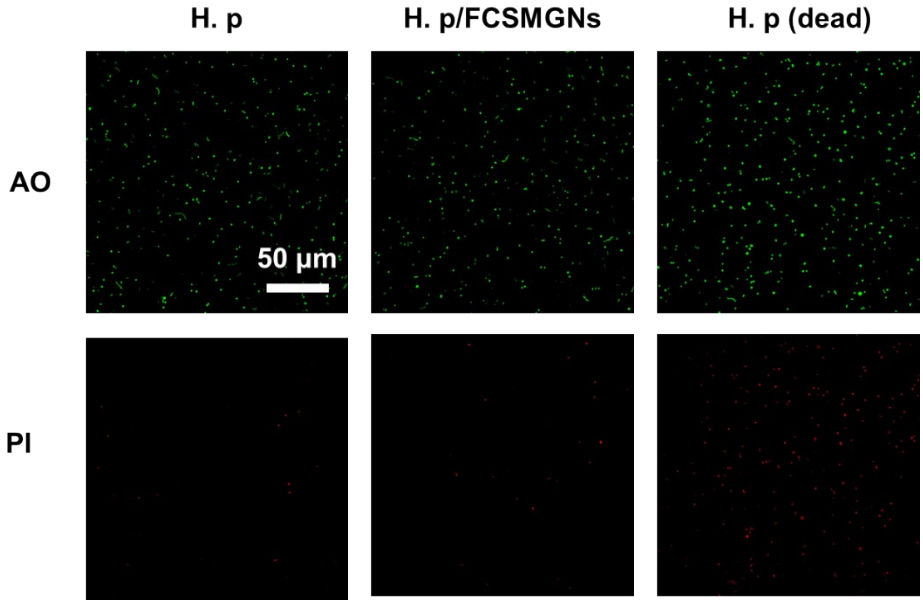

Figure S5. Evaluation of the *H. pylori* toxicity of FCSMGNs using a bacterial live/dead assay, PI-stained dead *H. pylori* (red), AO-stained all *H. pylori* (green).

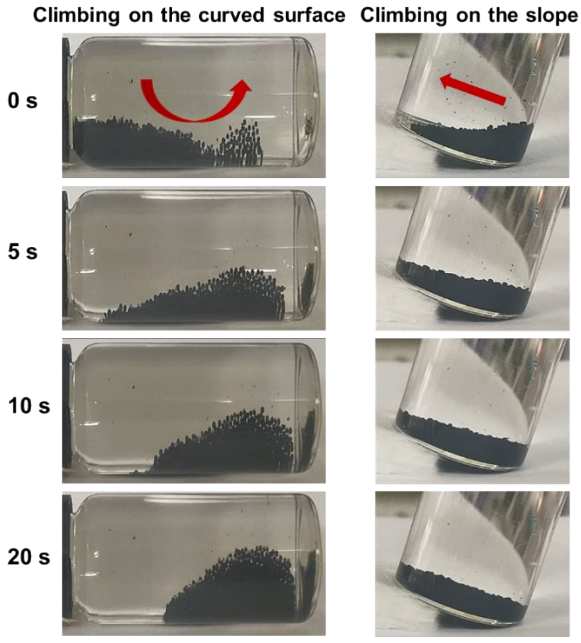

Figure S6. The climbing motion of the mircosweepers on the curved surface and the sloping underside of the glass bottle.

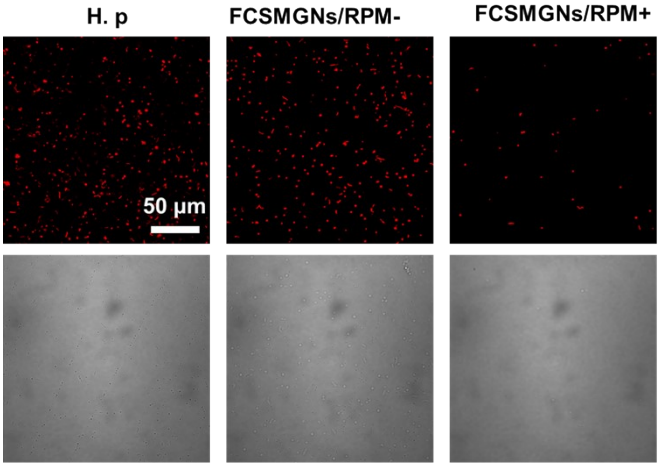

Figure S7. The confocal laser scanning microscopy (CLSM) images revealing the *H. pylori* capture ability of the FCSMGNs in SGF with or without the application of RPM, *H. pylori* was labeled by Cy5 (red).

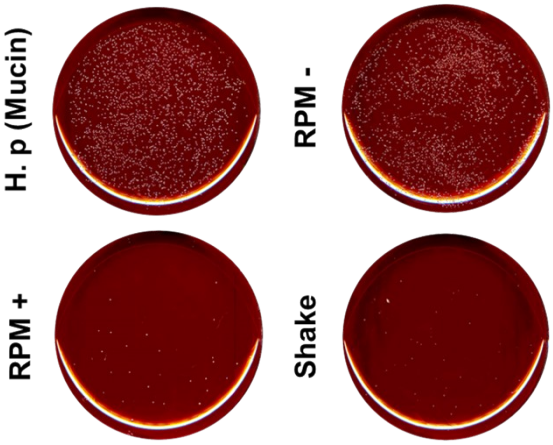

Figure S8. Photographs of *H. pylori* colonies treated with FCSMGNs in gastric mucus with or without the application of the RPM.

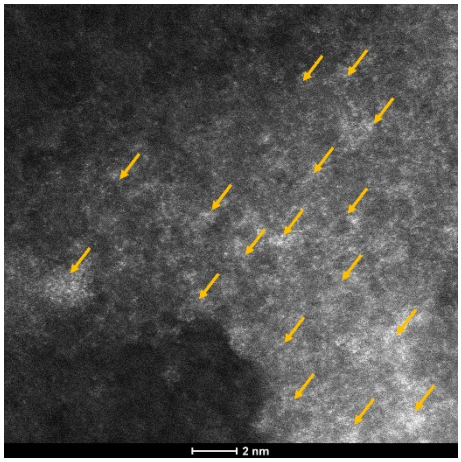

Figure S9. The high-resolution HAADF-STEM image of the hemin after pyrolysis, the

yellow arrows indicate Fe aggregates.

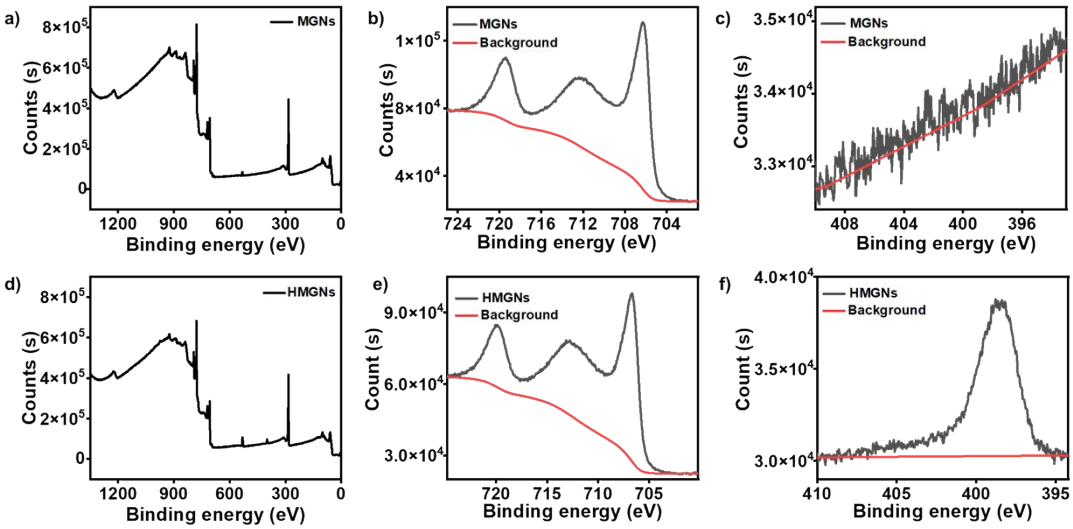

Figure S10. (a) XPS survey spectra of MGNs. High-resolution XPS spectra of (b) Fe 2p3 and (c) N 1s for MGNs. (d) XPS survey spectra of HMGNs. High-resolution XPS spectra of (e) Fe 2p3 and (f)N 1s for HMGNs.

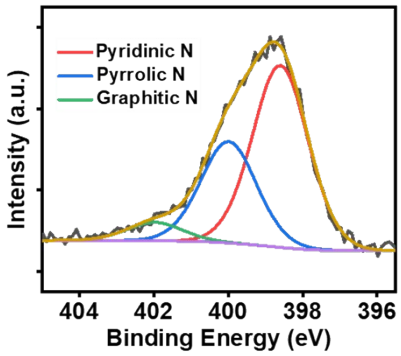

Figure S11. High-resolution XPS spectra of N 1s for HMGNs.

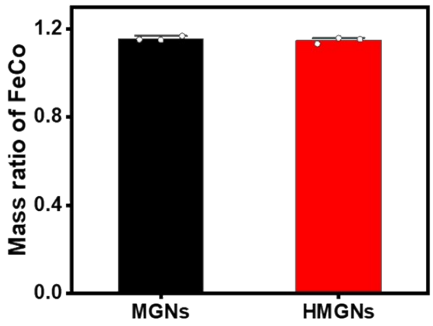

Figure S12. The Fe/Co mass ratio of MGNs and HMGNs was examined using ICP-OES.

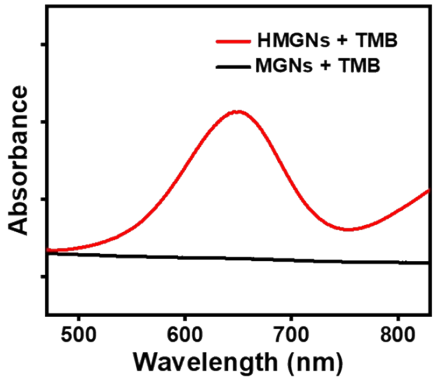

Figure S13. The UV-Vis absorbance spectra of TMB treated by MGNs and HMGNs in a pH 4.0 phosphate solution.

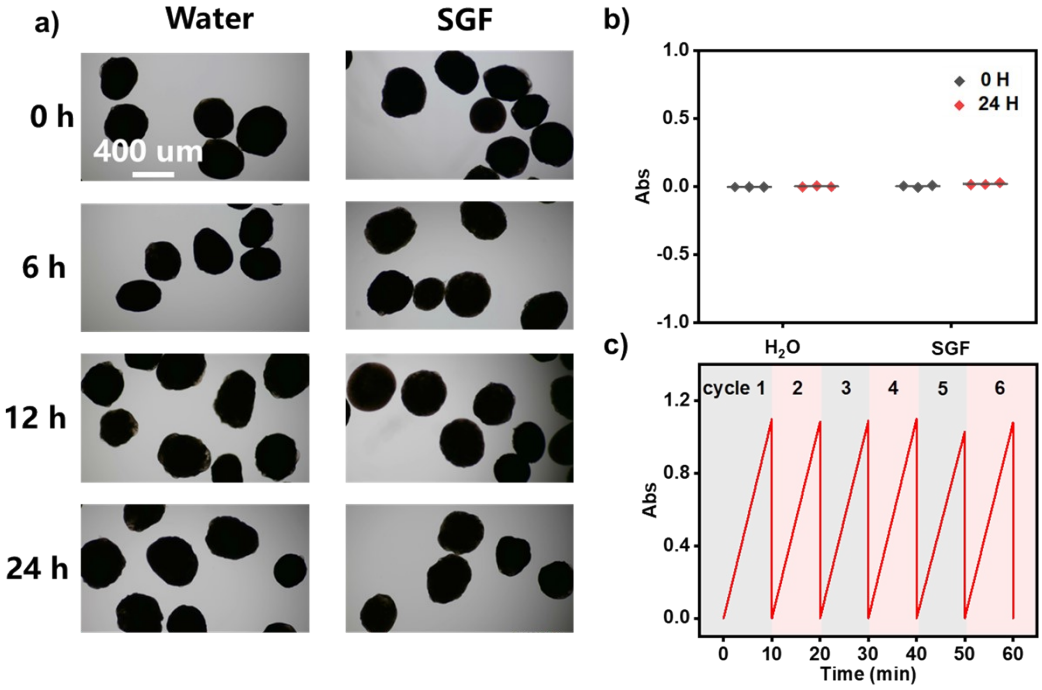

Figure S14. (a) Microscopy images of FCSHMGNs during 24-hour incubation with water and SGF. (b) The UV-Vis absorbance at 280 nm of the 24-hour supernatant from Figure S14a. (c) The catalytic ability of FCSHMGNs in six cycles.

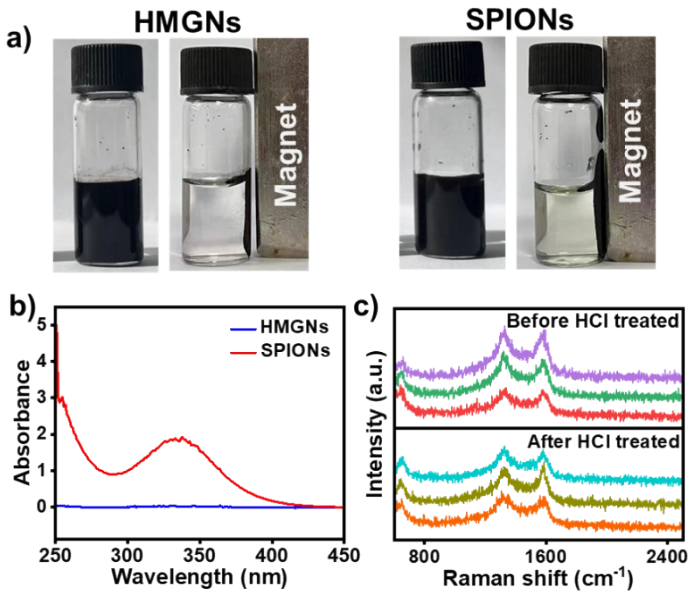

Figure S15. (a) photographs of HMGNs and SPIONs after 24-hour incubation with 1M HCl and the magnetic response under the application of magnet. (b) The UV-Vis spectra of the 24-hour supernatant from Figure S15a. (c) The Raman spectra of HMGNs before and after HCl treatment. It is obvious that the supernatant color of SPIONs after HCl treatment was light yellow, which may be caused by the iron ion leakage. Therefore, we further determined the UV-Vis spectra of the 24-hour supernatant of HMGNs and SPIONs after HCl treatment. Result showed that HMGNs possessed excellent stability in acid environment. On the contrary, SPIONs was destroyed by the HCl and produced iron ions (Fe^{3+}), which result in the peak around 333 nm. Moreover, the Raman spectra of HMGNs before and after HCl treatment also indicated the stability of the graphitic shell of HMGNs.