

Supporting information

## **Electro-responsive Supramolecular Graphene Oxide Hydrogels for Active Bacteria Adsorption and Removal**

*Bin Xue,<sup>†</sup> Meng Qin,<sup>†</sup> Junhua Wu,<sup>‡</sup> Dongjun Luo,<sup>⊥</sup> Qing Jiang,<sup>‡</sup> Ying Li\*,<sup>//</sup> Yi  
Cao\*,<sup>†</sup> and Wei Wang\*<sup>†</sup>*

<sup>†</sup> National Laboratory of Solid State Microstructure, Department of Physics, Nanjing University 22 Hankou Road, Nanjing, Jiangsu, China

<sup>‡</sup> Jiangsu Key Laboratory of Molecular Medicine, Medical School, Nanjing University, 22 Hankou Road, Nanjing, Jiangsu, 210093, P.R. China

<sup>//</sup> Jiangsu Engineering Technology Research Centre of Environmental Cleaning Materials, Jiangsu Key Laboratory of Atmospheric Environment Monitoring and Pollution Control, Jiangsu Joint Laboratory of Atmospheric Pollution Control, Collaborative Innovation Center of Atmospheric Environment and Equipment Technology, School of Environmental Science and Engineering, Nanjing University of Information Science & Technology, 219 Ningliu Road, Nanjing, Jiangsu, 210044, P.R. China

<sup>⊥</sup> Department of Hepatobiliary Surgery, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, 321 Zhong Shan North Road, Nanjing, Jiangsu, 210008, P.R. China

E-mail: caoyi@nju.edu.cn, yingliubc@gmail.com or wangwei@nju.edu.cn

## Experimental methods:

**Materials:** Tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate, dichlorotris(1,10-phenanthroline)ruthenium(II) hydrate and cytotoxicity detection kit (MTT and LDH) were purchased from Sigma-Aldrich. Tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) dichloride was purchased from Aladdin. GO was received as a generous gift from the Yu Lab in USTC. All chemicals were used without further purification.

**The accumulated bacteria removal efficiency:** The sample hydrogel was used for catch-and-release cycles (See above section for experimental details) in the same bacteria solution for three times to estimate the accumulated bacteria removal efficiency. The percentage of adsorbed bacteria was calculated as the change of the OD<sub>600 nm</sub> after three cycles of purification divided by the initial OD<sub>600 nm</sub> of the solution.

**The growth activity of the bacteria:** The adsorbed bacteria and bacteria with electric treatment were rinsed off from the hydrogel with PBS. Both *E.coli* and *SAU* were incubated in the LB media at 37 °C in orbital shakers at 225 rpm. OD at 600 nm was measured to monitor the growth of bacteria.

**The toxicity of GO and Ru(II) to different bacteria:** The bacteria (*E. coli* or *SAU*) solution (5 µL, OD<sub>600</sub>=0.6) was added into the LB solutions (5 mL) which contained different concentrations of GO or Ru(II). Control groups without GO and Ru(II) were also prepared. All the groups were placed in orbital shakers at 37 °C for 12 h. The concentrations of bacteria were monitored by OD at 600 nm. The percentage of bacteria viability was calculated as follows:  $\frac{A_{drug}-A_{blank}}{A_{control}-A_{blank}} \times 100\%$ .

**In vivo toxicity tests:** Four-week-old female ICR mice (Laboratory Animal Center of the Academy of Military Medical Science, Beijing, China) were used in *in vivo* toxicity tests. The animal experiments were conducted according to the Regulation on Experimental Animals of Nanjing University. All the treated mice were randomly divided into 6 groups (n=5). The solubility of Ru(II) in water is 40 mg mL<sup>-1</sup> and the maximum volume can be used for a mouse is 500 µL/20 gBW. So we used 500

$\mu\text{L}/20\text{gBWV}$  dose of  $40\text{ mg mL}^{-1}$  Ru (II) solution for the vivo toxicity test. The control groups (i.g. with water) and experiment groups (i.g. with Ru(II)) were killed by breaking their necks and dissected at the time as indicated in Figure S22.

**The adsorption of LX2 cells with GO/Ru(II) and GO/Ru(III) hydrogel:** These cells were grown in RPMI 1640 medium with 10% FBS in the presence of  $100\text{ U mL}^{-1}$  penicillin and  $100\text{ }\mu\text{g mL}^{-1}$  streptomycin and maintained at  $37\text{ }^{\circ}\text{C}$  in a humidified atmosphere containing 5% (v/v)  $\text{CO}_2$ . The hydrogel were added into the cell suspensions and the number of cells was directly counted before and after the adsorption process under the field of an optical microscope (LWD300-38LT, Cewei, China).

**The cytotoxicity of GO and Ru(II) in LX2 and 3T3 cells:** LX2 and 3T3 cells were seeded in a 24-well plate at a density of  $1 \times 10^4$  cells per well (with 1.0 mL DMEM medium). After 24 h incubation, 20  $\mu\text{L}$  of GO or Ru(II) solution was added to the wells with cells. Then all the cells were cultured for additional 48 h. The cell viability was assessed by the MTT method. Firstly, cells were incubated with MTT ( $0.5\text{ mg mL}^{-1}$ ) for 4 h at  $37\text{ }^{\circ}\text{C}$ . During this incubation period, water-insoluble formazan crystals were formed, which were dissolved by the addition of 100  $\mu\text{L}$  DMSO per well. The optical densities at 570 nm were measured using an enzyme-linked immunosorbent assay plate reader. Wells containing culture medium and MTT but no cells acted as blanks. The percentage of cell viability was calculated as follows:

$$\frac{A_{\text{drug}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%.$$

**The LDH release measurement:** The LDH released from bacteria was measured using a standard LDH Cytotoxicity Assay Kit (Sigma-Aldrich). The desorbed bacteria and electrically deactivated bacteria suspensions were centrifuged at a speed of 6000 rpm for 5 min and then the supernatant was taken out and mixed with the testing solution (containing 2-*p*-iodophenyl-3-nitrophenyl tetrazolium chloride, diaphorase and lactic acid) at the volume ratio of 2:1 (supernatant: the testing solution). The bacteria solution treated with 10% (v/v) LDH releasing agent was used as the positive control and prepared with the same procedure as that for the desorbed

bacteria and electrically deactivated bacteria. All solutions were incubated for 1 h at 37 °C in an orbital shaker.

Then a V-550 (JASCO Inc., Japan) spectrophotometer was used to quantify the released LDH by monitoring the UV-Vis absorbance at 490 nm.

**Scanning electron microscope (SEM):** SEM images were obtained using a Quanta Scanning Electron Microscope (Quanta 200, FEI) at 20 kV. The hydrogels were lyophilized prior to the measurement.

**UV-Vis spectroscopy:** UV-Vis spectra of all samples were recorded using a V-550 (JASCO Inc., Japan) spectrophotometer. The cuvette width was 1 cm and the bandwidth was set as 0.2 nm.

**Fluorescence spectroscopy:** Spectrofluorometric experiments were performed using an FP-6500 (JASCO Inc., Japan). The emission spectra were measured using an excitation wavelength of 365 nm.

**Mechanical Measurements:** The hydrogels were carefully transferred to the rheometer plate of the Thermo Scientific Haake RheoStress 6000 with a spatula prior to the measurement. The rheology experiments were then carried out using a strain-sweep mode with a strain amplitude range of 0.01% to 100% at 1 Hz as shown in Figure S2 and a frequency-sweep mode with frequency of 0.01 Hz to 100 Hz at 0.1% strain as in Figure 2 (geometry: 1°/20 mm of cone and plate; gap: 0.37 mm; temperature: 20 °C). The variable temperature experiments were carried out in the temperature mode at strain of 0.1% and frequency of 1 Hz.

**Cyclic voltammogram (CV):** A 3-electrode system was used to obtain cyclic and linear sweep voltammograms; a glassy carbon electrode (working electrode), Ag/AgCl (reference electrode), and a platinum wire (counter electrode) were connected to an electrochemical workstation with a scan rate of 100 mV s<sup>-1</sup>. The samples were GO/Ru hydrogels including GO/Ru(II) and GO/Ru(III) as well as the GO solution. The concentrations of Ru and GO in the samples were 0.025 mg mL<sup>-1</sup> and 0.25 mg mL<sup>-1</sup>.

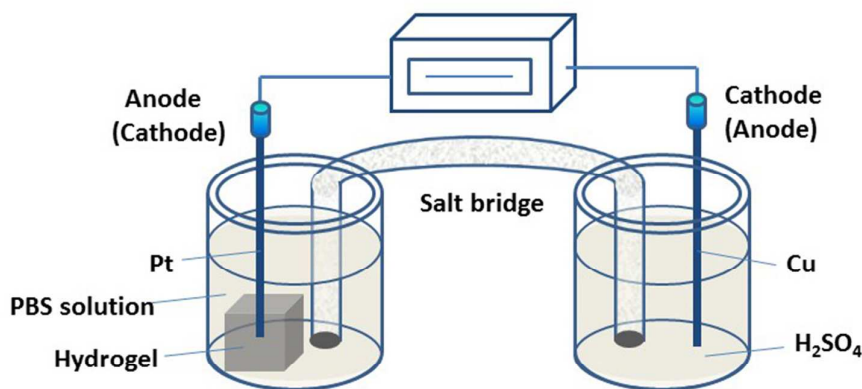
**Chronoamperometry:** Chronoamperometry also used the 3-electrode system as the CV experiments. The volumes of GO/Ru(II) and GO/Ru(III) hydrogels were both 0.4

cm<sup>3</sup>. The potential during the oxidation and reduction were +2 V and -1.2 V, respectively.

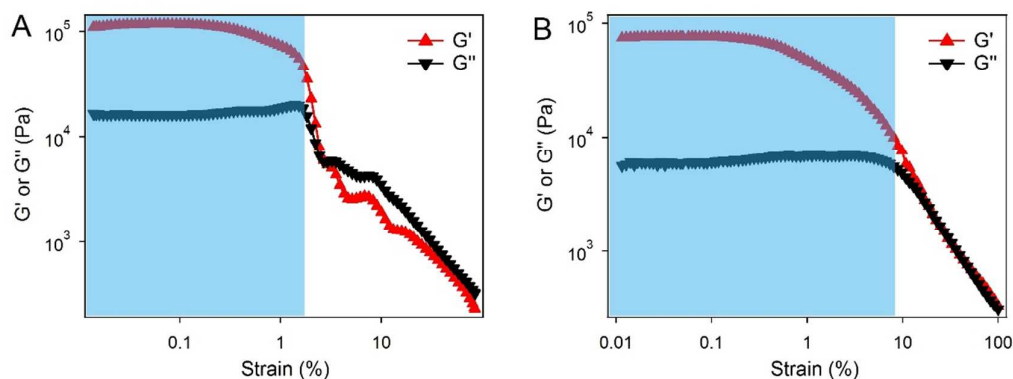
**Zeta potential:** The zeta potential measurements were conducted in PBS buffer, the same buffer as we used for bacteria catch and release experiments, to minimize the effect of pH, ionic strength and other factors on the measured zeta potentials. Typically, 100 uL of hydrogels were suspended in 900 uL of PBS buffer and fractured by a vortex mixer (Scientific Instruments, USA) for 15 min at its maximum power. Then the zeta potential of the hydrogel suspension was measured by the Zetasizer Nano ZS (Malvern, UK). The same hydrogels were measured for at least three times to guarantee the reproducibility.

**The test of bacteria-killing effects using agar plates:** Agar plates were prepared and inoculated with 150  $\mu$ L of different bacteria solutions before and after electric deactivation. Two different bacteria concentrations ( $OD_{600}=0.6$  and  $OD_{600}=0.2$ ) were used for the experiments. The agar plates were allowed to grow for 12 h at 37 °C in an incubator and then imaged.

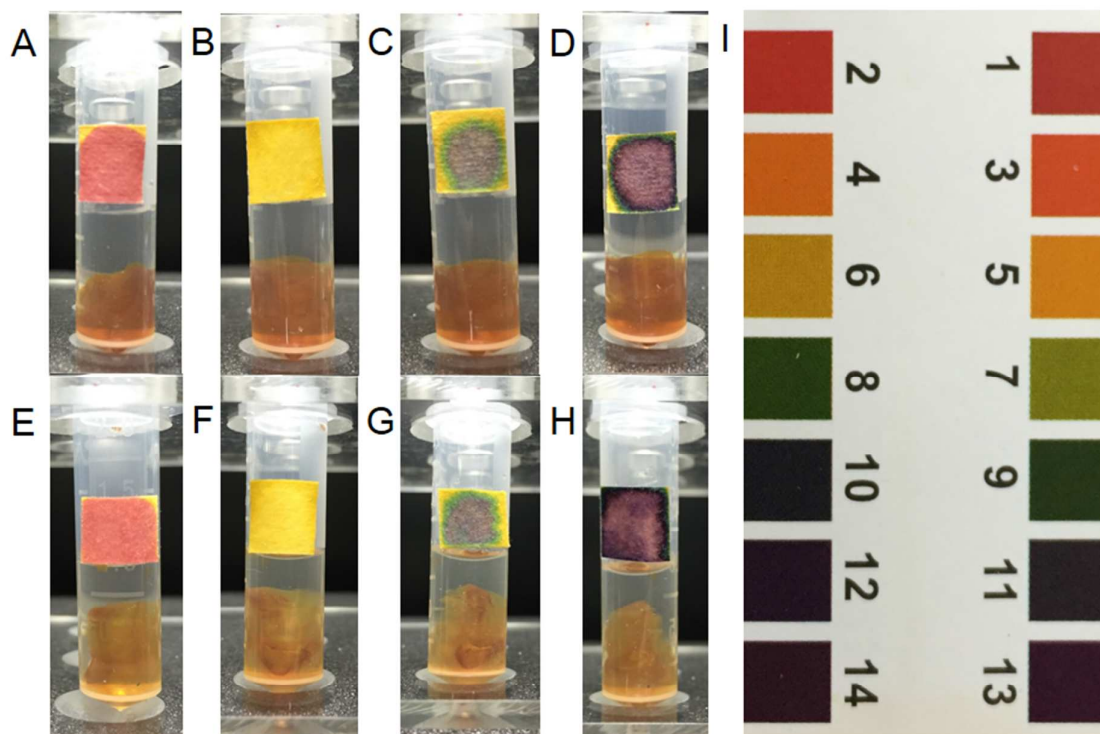
**FT-IR measurement for the hydrogels:** All the FT-IR samples were prepared by lyophilization of the hydrogels prior to the measurement. FT-IR data was recorded in a Fourier Transform Infrared spectrometer (NEXUS870, NICOLET).



**Figure S1.** Schematic diagram of the electrochemical cell for the electro-oxidation of the hydrogel.

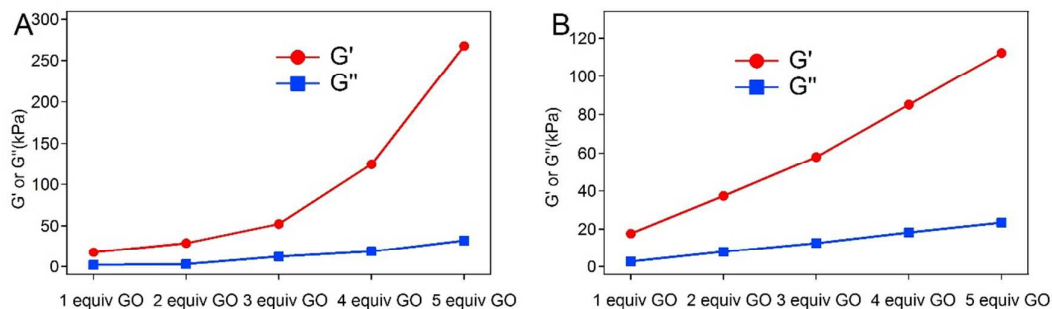


**Figure S2.** The strain sweep experiments for the GO/Ru hydrogels. (A) The strain-sweep experiment of the GO/Ru (II) at a constant sweep frequency of 1 Hz. (B) The strain-sweep experiment of the GO/Ru (III) hydrogel at a constant sweep frequency of 1 Hz. The linear viscoelastic (LVE) regions are highlighted in light blue.

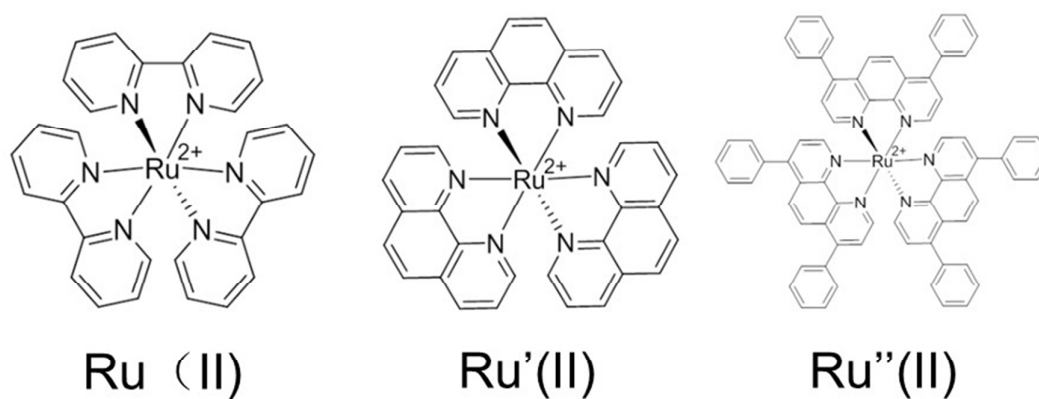


**Figure S3.** The stability of GO/Ru hydrogels against different pHs. The solution was PBS adjusted by HCl and NaOH. GO/Ru(II) hydrogel (A-D) and GO/Ru(III) hydrogel

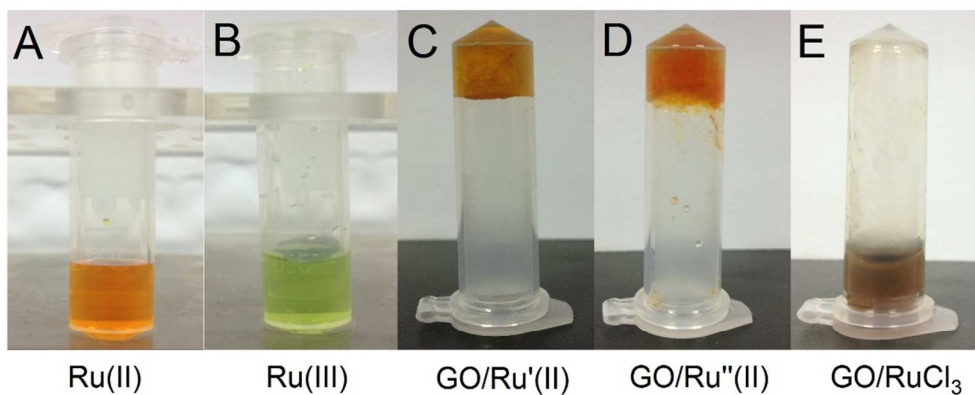
(E~H) were immersed in solutions at different pH (2~14). I is a pH test standard colorimetric card.



**Figure S4.** (A) The rheological properties of the hydrogels with different amounts of GO while the concentration of Ru(II) remained  $0.25 \text{ mg mL}^{-1}$ . 1 equiv GO means  $0.6 \text{ mg mL}^{-1}$  GO. (B) The rheological properties of the hydrogels with different amounts of Ru (II) while the concentration of GO was  $2.5 \text{ mg mL}^{-1}$ , and 1 equiv Ru(II) is  $0.06 \text{ mg mL}^{-1}$ .

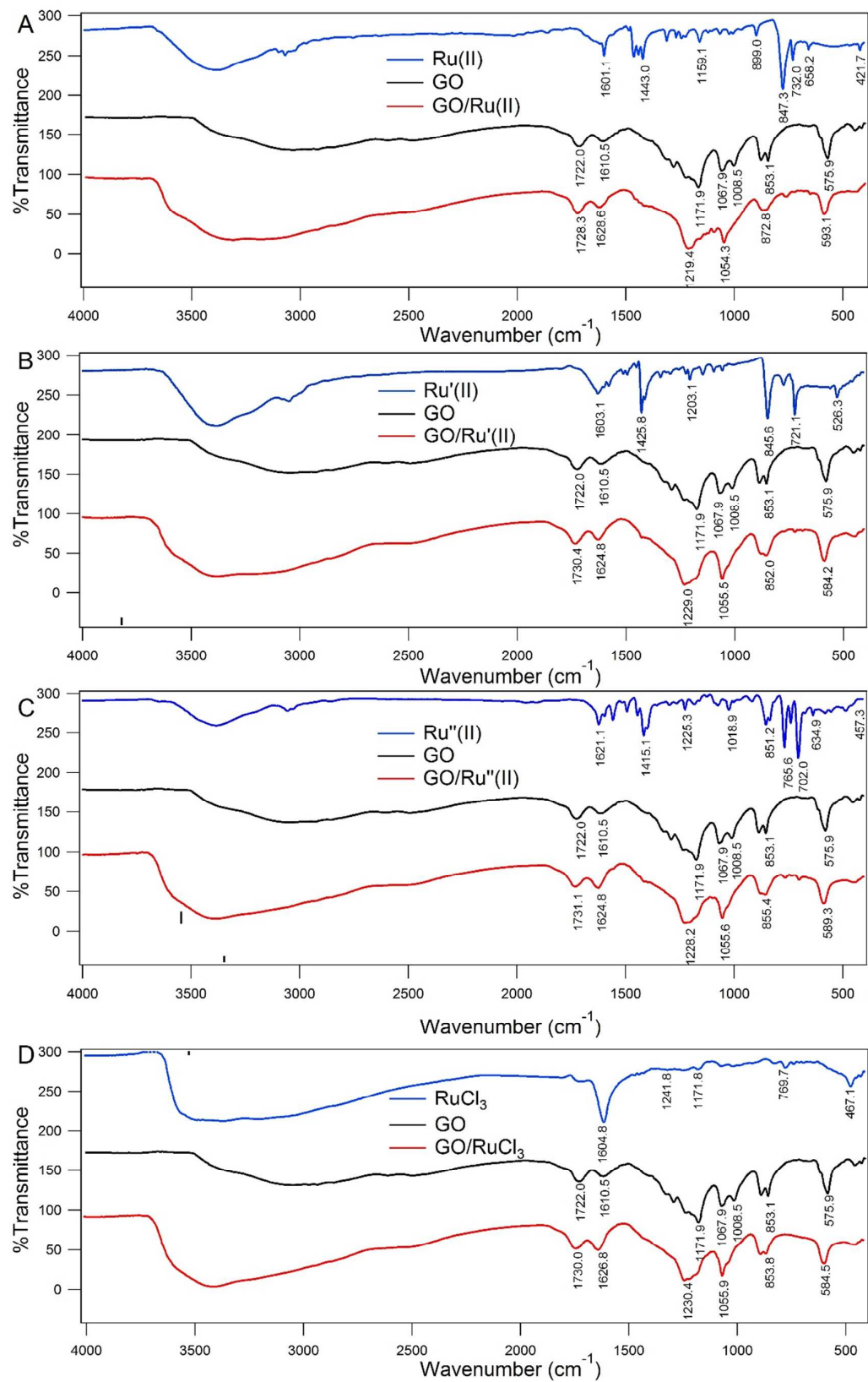


**Figure S5.** Chemical structures of ruthenium (II) complexes.

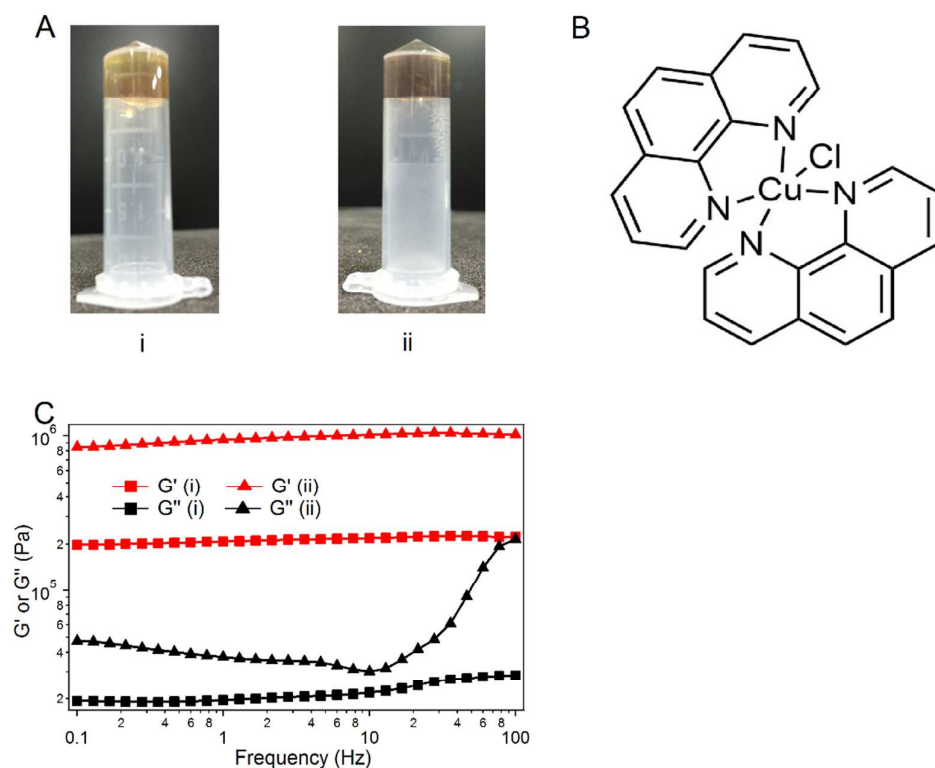


**Figure S6.** Images of Ru solutions and GO/Ru hydrogels. (A) Ru (II) solution ( $1 \text{ mg mL}^{-1}$ ); (B) Ru(III) solution ( $1 \text{ mg mL}^{-1}$ , oxidized from Ru(II) solution by electrolysis); (C) GO/Ru'(II); (D) GO/Ru''(II); and (E) GO/RuCl<sub>3</sub>.

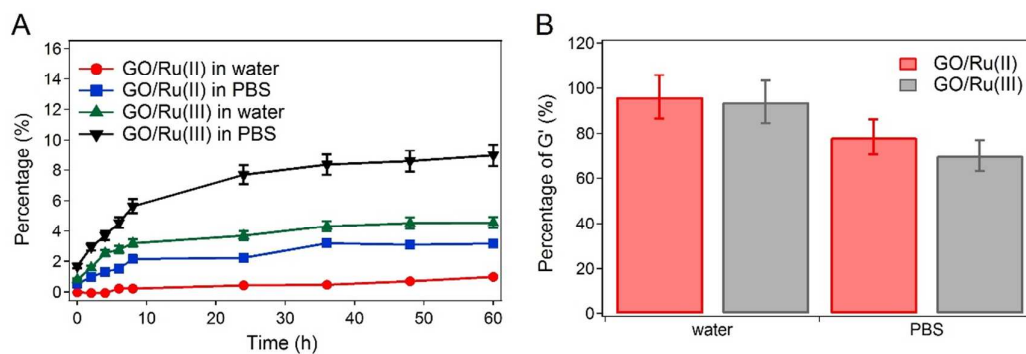




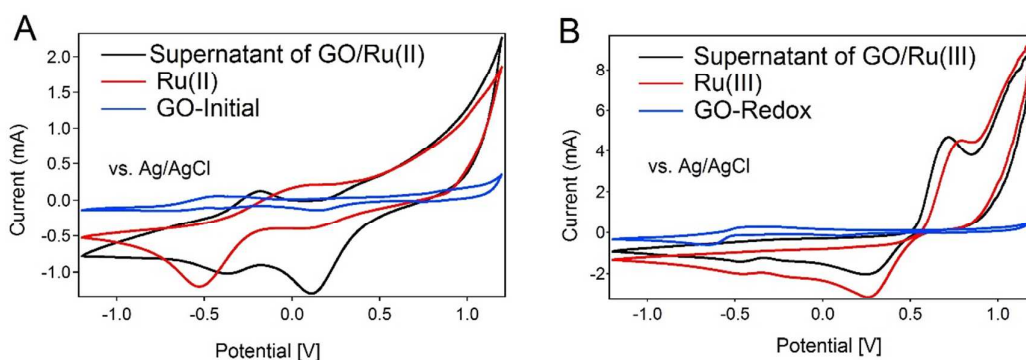
**Figure S7.** FT-IR of GO, ruthenium complex and the mixtures of both. The peaks in the range of  $600\text{ cm}^{-1}$  and  $800\text{ cm}^{-1}$  from the vibration of the ligands in Ru, Ru' and Ru'' are disappeared when interacting with GO.



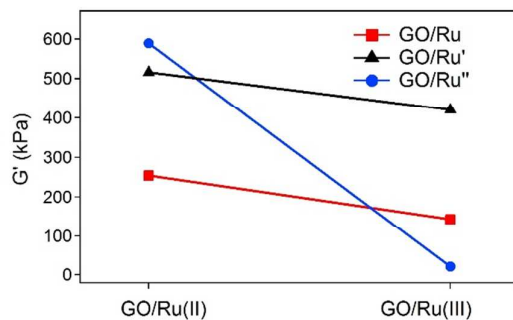
**Figure S8.** Hydrogels formed by GO and a copper complex. (A) Optical graphs of GO/Cu(II) hydrogel. The concentrations of GO were  $2.5\text{ mg mL}^{-1}$  (i) and  $5\text{ mg mL}^{-1}$  (ii). The concentrations of copper complex were all  $0.25\text{ mg mL}^{-1}$ . (B) Chemical structure of the copper complex. (C) The rheological properties of GO/Cu(II) at 0.1% strain in the frequency range of 0~100 Hz.



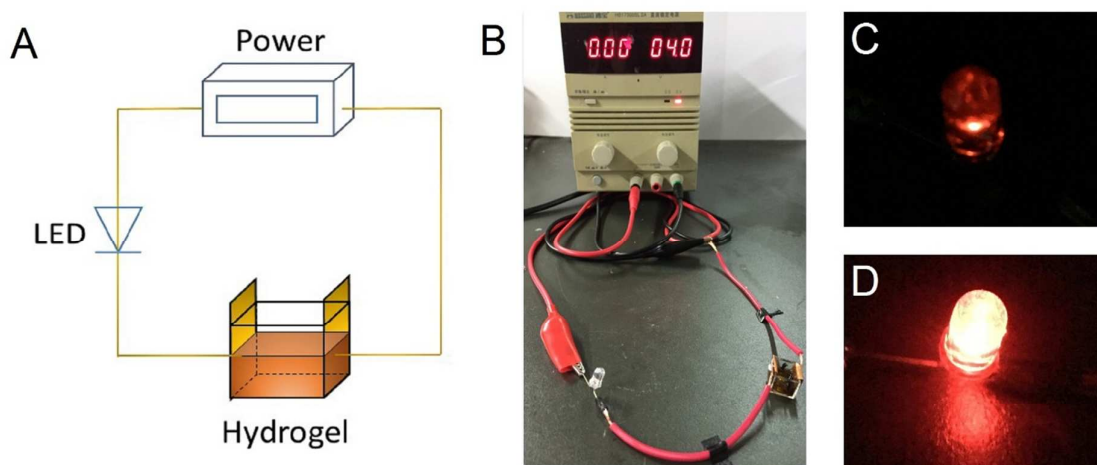
**Figure S9.** The ion release and mechanical properties change of the GO/Ru(II) and GO/Ru(III) in 60 h. (A) The ruthenium ion release from the hydrogel in a time period of 60 h in PBS and deionized water. (B) The normalized mechanical properties of GO/Ru(II) and GO/Ru(III) hydrogels after 60-h incubation in PBS and deionized water compared to the original GO/Ru(II) and GO/Ru(III) hydrogels.



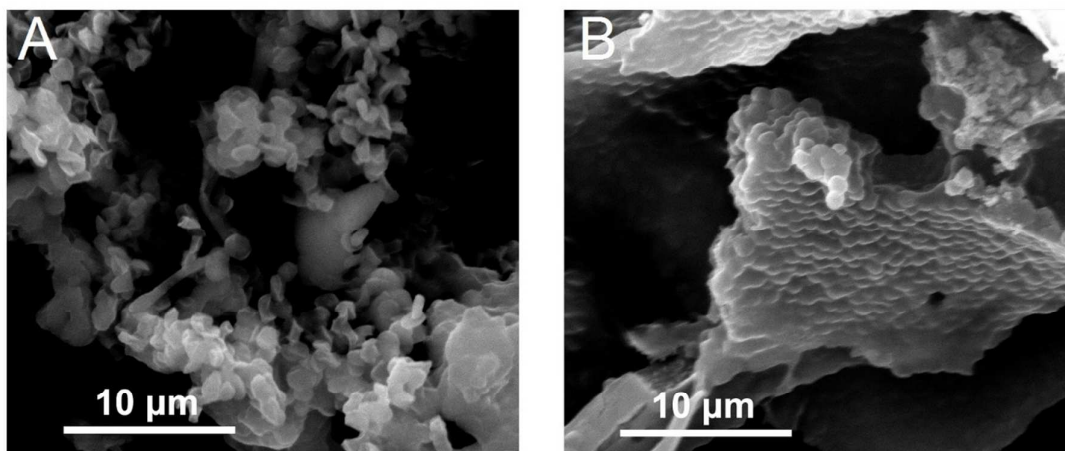
**Figure S10.** Cyclic voltammograms of the hydrogels. (A) Typical cyclic voltammograms of Ru(II), supernatant of GO/Ru(II) hydrogel and the initial GO solution. (B) Typical cyclic voltammograms of Ru(III), supernatant of GO/Ru(III) hydrogel and the GO solution after being electrolyzed once.



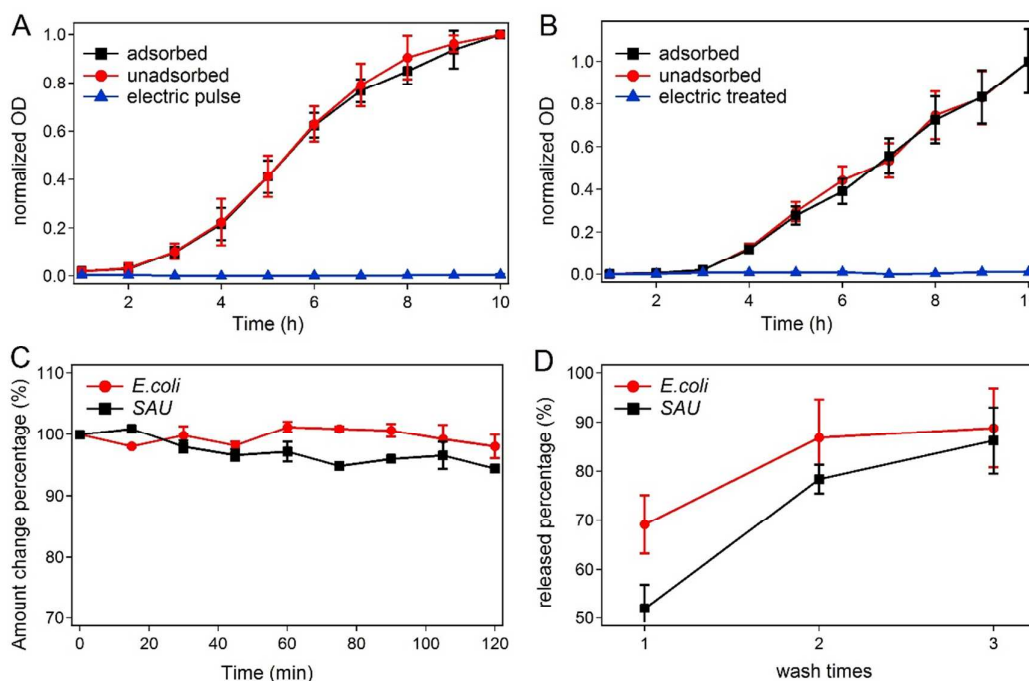
**Figure S11.** Rheological properties change using different ruthenium complexes. The concentration of GO was  $2.5 \text{ mg mL}^{-1}$  and that of ruthenium was  $0.25 \text{ mg mL}^{-1}$ .



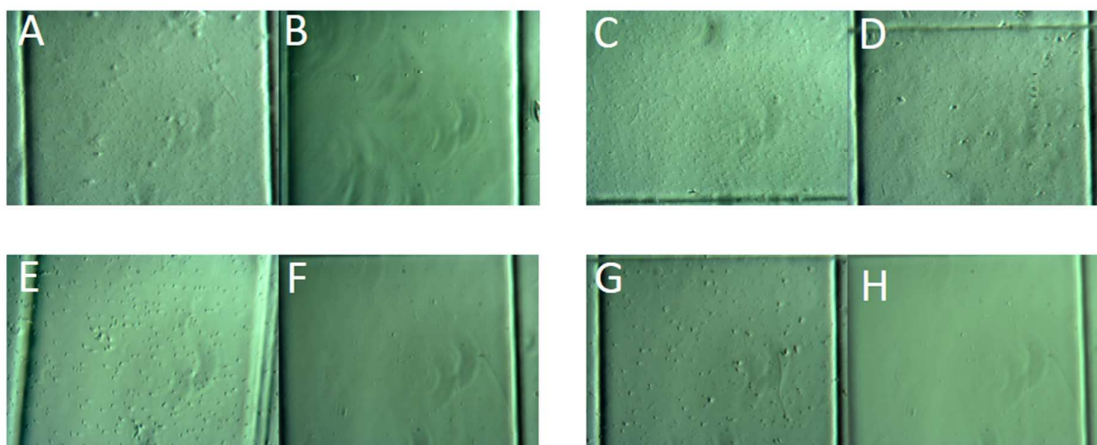
**Figure S12.** GO/Ru hydrogel as a controllable resistor. (A) The schematic of the electric circuit. An LED light bulb is used to visualize the change of the current in the circuit. (B) The optical image of the electric circuit. (C) and (D) The optical images of the LED bulb taken in a dark room using GO/Ru(II) and GO/Ru(III) as the controllable resistors, respectively.



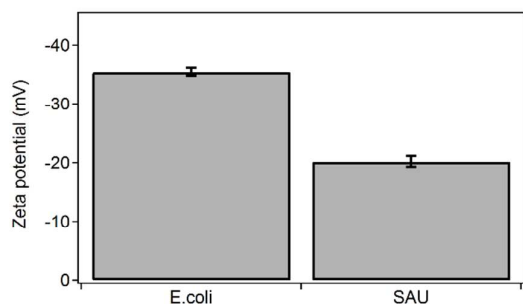
**Figure S13.** (A) SEM images of *E. coli*. (B) SEM images of *SAU*.



**Figure S14.** (A) The growth curves of the *E. coli* adsorbed and unadsorbed by the GO/Ru(II) hydrogel as well as the *E. coli* treated with electric pluses. (B) The growth curves of the *SAU* adsorbed and unadsorbed by the GO/Ru(II) hydrogel as well as the *SAU* treated by electric pluses. (C) The growth of both *E. coli* and *SAU* in PBS during a period of two hours. (D) The percentages of released *E. coli* and *SAU* after different wash times.

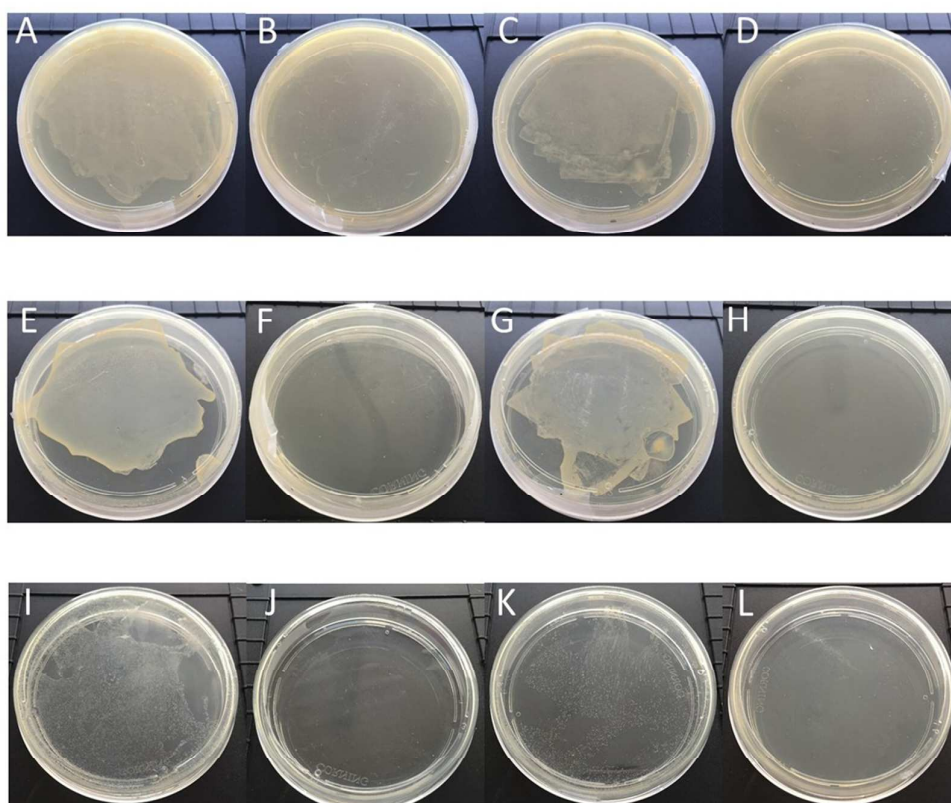


**Figure S15.** Microscopic images of hydrogels adsorbing *E. coli*. *E. coli* solution before (A) and after (B) adsorption using GO/Ru(II). (82% *E. coli* were removed.) The microscopic pictures of the *E. coli* solution before (C) and after (D) adsorption using GO/Ru(III). (5% *E. coli* were removed.) The microscope pictures of the *SAU* solution before (E) and after (F) adsorption using GO/Ru(II). (92% *SAU* were removed.) The microscopic pictures of the *SAU* solution before (G) and after (H) adsorption using GO/Ru(III). (95% *SAU* were removed.)

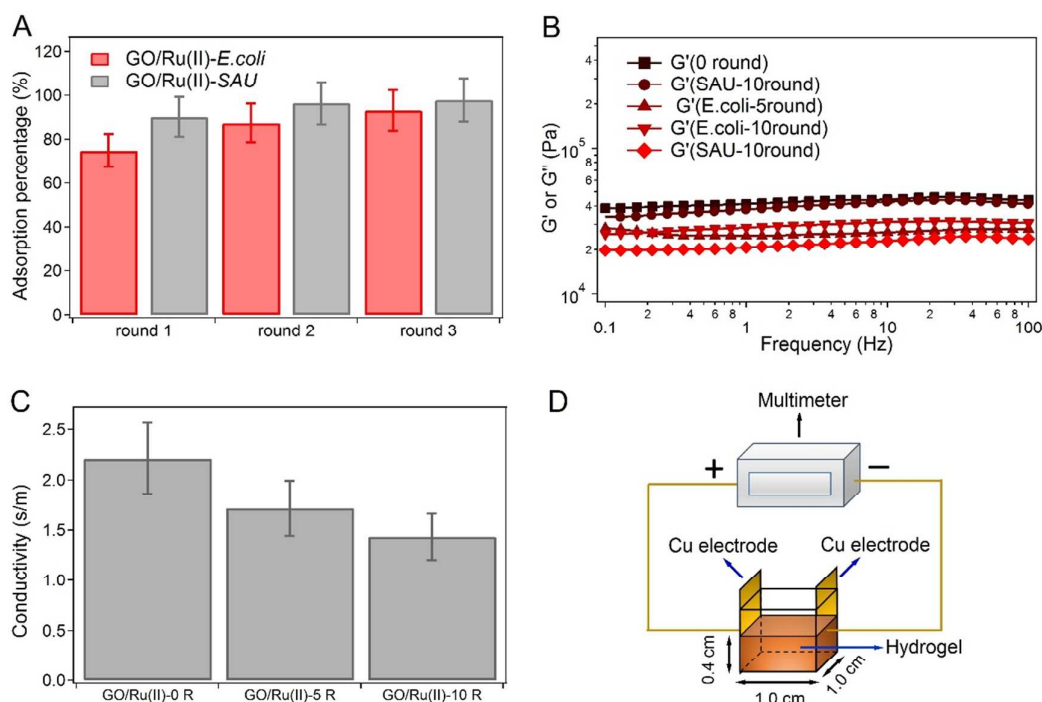


**Figure S16.** Zeta potentials of the *E. coli* and *SAU*.



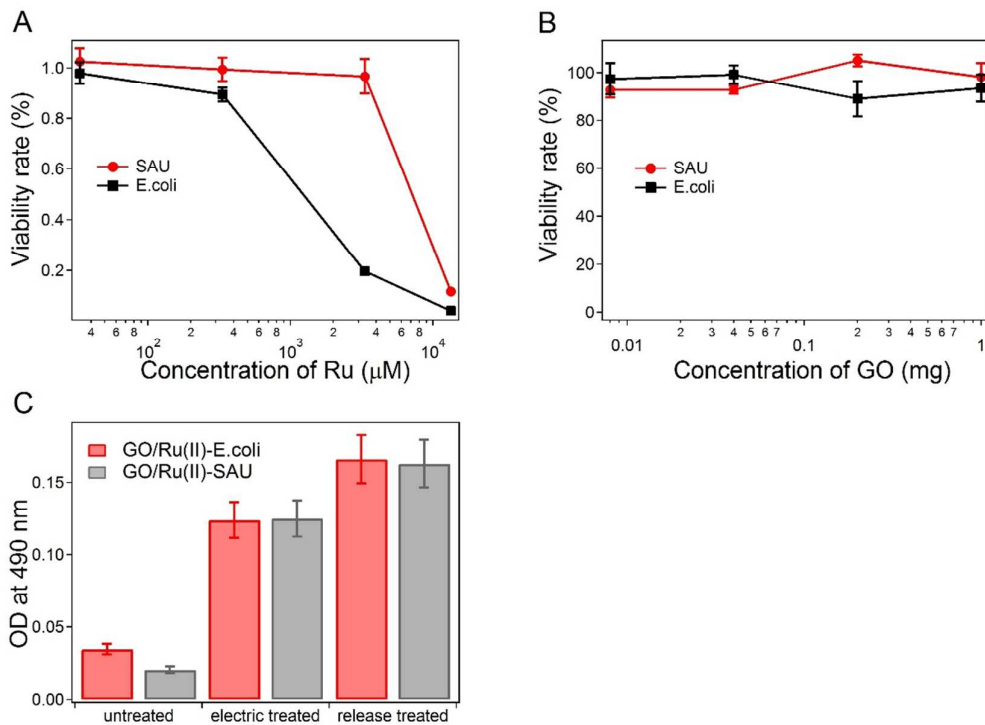


**Figure S17** Aerobic plate count (APC) experiments to measure the deactivation efficiency by the electric treatment. Agar plates were prepared and inoculated with 150  $\mu$ L of different bacteria solutions before and after electric deactivation. (A) Untreated *E. coli* at the OD<sub>600</sub> of 0.6. (B) Electrically treated *E. coli* at the OD<sub>600</sub> of 0.6. (C) Untreated *E. coli* at the OD<sub>600</sub> of 0.2. (D) Electrically treated *E. coli* at the OD<sub>600</sub> of 0.2. (E) Untreated *SAU* at the OD<sub>600</sub> of 0.6. (F) Electrically treated *SAU* at the OD<sub>600</sub> of 0.6. (G) Untreated *SAU* at the OD<sub>600</sub> of 0.2. (H) Electrically treated *SAU* at the OD<sub>600</sub> of 0.2. (I) Untreated *E. coli* which initial OD<sub>600</sub> was 0.6 and diluted by 1000 times. (J) Electrically treated *E. coli* which initial OD<sub>600</sub> was 0.6 and diluted by 1000 times. (K) Untreated *SAU* at the OD<sub>600</sub> which initial OD<sub>600</sub> was 0.6 and diluted by 1000 times. (L) Electrically treated *SAU* which initial OD<sub>600</sub> was 0.6 and diluted by 1000 times.

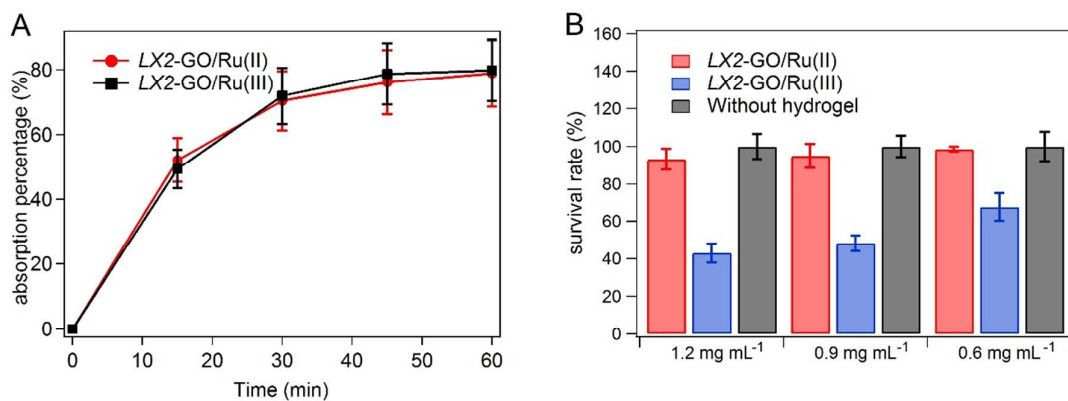


**Figure S18.** The reusability of the hydrogel for multiple runs of purification. (A) The accumulated bacteria removal efficiency during three runs of purification. (B) The mechanical properties of GO/Ru(II) hydrogel after five and ten rounds of *E. coli* and *SAU* adsorption. (C) The conductivities of GO/Ru(II) hydrogel after 0, 5 and 10 rounds of adsorption. (D) The schematic of the device used to measure the conductivity. The hydrogel was sandwiched between two copper layered electrodes and the volume of the hydrogel was 0.4 mL.

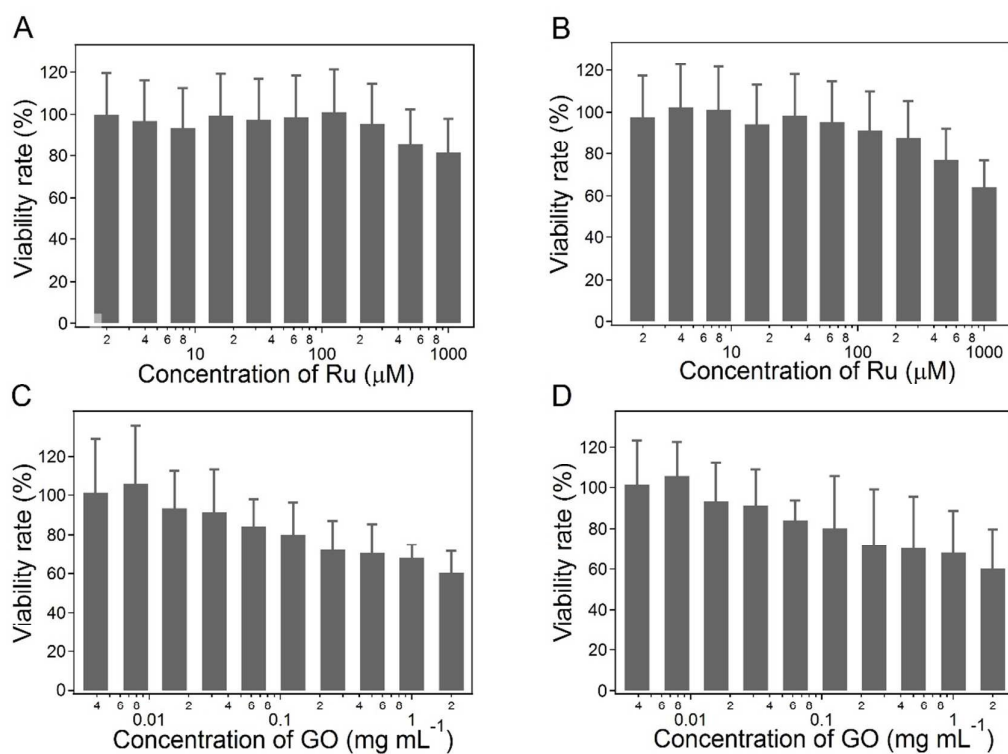




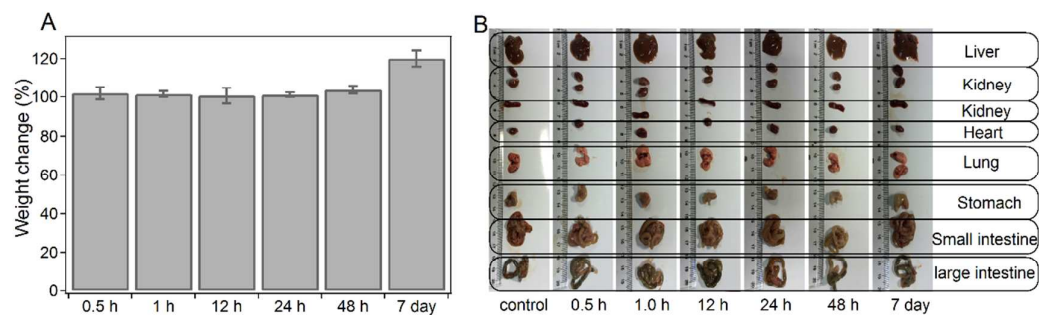
**Figure S19.** (A) The toxicity of Ru(II) complex to *E. coli* and *SAU*. (B) The toxicity of GO solution to *E. coli* and *SAU*. (C) The LDH release of untreated bacteria, electrically treated bacteria and bacteria treated with LDH releasing agent, in which LDH was fully released (label as release treated).



**Figure S20** (A) The *LX2* adsorption kinetics for GO/Ru(II) and GO/Ru(III) hydrogels monitored by cell counting. (B) The survival rate of *LX2* cells with and without hydrogels.



**Figure S21.** Cytotoxicity of Ru(II) and GO to cells. (A) The cytotoxicity of Ru (II) solution to *LX2*. (B) The cytotoxicity of Ru(II) solution to *3T3*. (C) The cytotoxicity of GO solution to *LX2*. (D) The cytotoxicity of GO solution to *3T3*.



**Figure S22.** (A) The weight change of mice at different time points after feeding with the ruthenium complex. (B) The optical photos of mice organs for the control group and experimental groups.