Preparation of Graphene Oxide (GO) /Lanthanum Coordination Polymers for the Enhancement of the Bactericidal Activity

Jia Wang[1, #], Zezhi Shan[2,3, #], Xiao Tan[1], Xinxiang Li[2, 3, *], Zhenqi Jiang[4, *], Jieling Qin[1,*]

1. Tongji University Cancer Center, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, Shanghai 200092, China

2. Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, China

3. Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China

4. Institute of Engineering Medicine, Beijing Institute of Technology, Beijing 100081, China

* Correspondence: 1149lxx@sina.com (Xinxiang Li) 7520200073@bit.edu.cn

(Zhenqi Jiang) and qinjieling770@hotmail.com（Jieling Qin）

# These authors contributed equally.

1. Materials

The sodium citrate ($NaNO_3$, AR) is obtained from Nanjing Chemical Reagent Co., Ltd. And the beef extract (BR) was purchased from Beijing Shuangxuan Microbe Medium Products Plant. The sulfuric acid (H_2SO_4, AR) and potassium permanganate ($KMnO_4$, AR) are received from Shanghai zhongshi Corporation. The PBS buffer is prepared using 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 and sterilized for further use. While the others such as graphite, sodium salicylate (SS), 8-Hydroxyquinoline (8-hq, AR), sodium peroxide and lanthanide chloride hydrates ($LaCl_3 \cdot 7H_2O$) are purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received without further purification.

Inoculum (*E. coli, S. aureus, B. subtilis, S. typhi, P. aeruginosa, K.pneumonia, B. pumilus*) are obtained by inoculating with a microbiological loop from original bacterium (American type culture collection, ATCC), after several time vortex, the inoculum are cultivated in the thermostatic water shaker for 24 h (*S. aureus* culture requires 48 h) [1].

2. Characterization

The morphologies of the as-synthesized products are examined by field-emission scanning electron microscopy (FESEM, JEOL, JSM-7001F), transmission electron microscopy (TEM, JEOL, JEM-2100) and atomic force microscopy (AFM, MFP-3D SA). UV-Vis spectra are recorded ranged from 200 to 600 using a Shimadzu UV2450 spectrometer. FT-IR spectra are recorded within 4000- 400 cm^{-1} wave number range by a Nicolet Nexus 470 IR spectrometer with the KBr pellet technique. X-ray photoelectron spectroscopy (XPS) measurements are carried out using a VG Scientific ESCA 2000 spectrometer (VG Microtech, UK) with an Mg-Kα X-ray source set at 170 W (13 mA and 13 kV). The fluorescence at different time points is measured with a fluorimeter (F-7000, HITACHI, Japan, 200-800 nm). Thermogravimetry properties (TGA) of the GLCPs nanocomposites are measured under nitrogen on a Netzsch STA 449C simultaneous thermal analyzer within 25°C to 1000°C.

3. Evaluation of bactericidal activities of samples

The bacterial liquid is standardized ($1*10^5$ -$1*10^7$ CFU/mL) by measuring the absorbance (A 625=0.1; SP-1800 Spectrophotometer, Pye-Unicam, Cambridge, UK.) using an optical density (OD). In order to get the zone of inhibition**,** the GLCP nanocomposites (100 μL, DMF as dissolvent) with a bacterial suspension (bacterium concentration range from 10^5 to 10^7 CFU/mL) cultured in nutrient broth (NB) is dropped in the oxford cup placed on a nutrient agar plate (Müller-Hinton agar or Sabouraud Dextrose agar)., the plate is given to observe the zone of inhibition, after the incubation at 37 °C for 48 h.

The minimum inhibitory concentration (MIC) indicates the lowest biomaterial concentration to inhibit the visible growth of bacterial. Specifically, the gradient concentrations of GLCPs is given in the tubes containing different amounts of bacteria and prepared in the shaker cultivation at 37 °C for 24 h for further detection. While the minimum bactericidal concentration (MBC) represents the minimum concentration of the sample to kill 99.9% of the bacterial. For the observation of MBC, proper amount of each culture medium with no visible growth is inoculated in agar plates. After 24 h aerobic incubation at 37 ℃ in the incubator, the number of surviving organisms is determined. Each experiment is repeated three times to confirm the MIC and MBC results.

In order to further investigate the bactericidal effect GLCP on the bacteria, the best sample GLCP-4 is chosen as the excellent antibacterial composite from the detection of zone of inhibition, MIC and MBC results and the flat colony counting method is harnessed for determination of the viable bacterial numbers after incubation. In a typical process, 200 ppm GLCP-4 is dispersed in the sterilized PBS solution, followed by the addition of 10^6 CFU/mL bacteria. All the tubes were afterwards incubated in a temperature-controlled rotary shaker at 20 °C for 0, 24 h, 48 h, respectively.

For the characterizations of bacteria before/after the treatment of samples, 10 μL of each specimen is loaded on TEM copper grids followed by staining with tungstophosphoric acid. After air-dry the copper grids, the samples are examined using the TEM (JEOL JEM-2100) as described above.

Table S1. The ratio of GO, lanthanum chloride and ligands for the preparation of GLCPs

	LCP	GLCP-1	GLCP-2	GLCP-3	GLCP-4	GLCP-5
GO (mg)	0	10	20	50	100	200

Table S2. The peaks of FT-IR spectra in GO, ligands and the GLCPs

Sample	GO					SS				8-hq				v_{RE-O}
	v_{O-H}	$v_{C=O}$	δ_{O-H}	v_{C-OH}	v_{C-O}	δ_{O-H}	v_{as}	v_s	$\triangle v$	v_{O-H}	$v_{C=O}$	$v_{C=N}$	δ_{O-H}	
GO	3000-3700	1740	1417	1228	1040									
SS						1490	1591	1375	216					
8-hq										3103	1093	1579	1223	
GLCP-1						1496	1593	1387	206	3090	1103	1572	1620	484
GLCP-5						1498	1594	1387	207	3087	1103	1572	1620	484

Table S3. The peaks of UV-Vis spectra in GO, ligands and the GLCPs

Sample	K absorption band	B absorption band
SS	249	340
8-hq	226	314
LCP	255	375
GLCP-1	262	377
GLCP-2	264	378
GLCP-3	266	379
GLCP-4	267	381
GLCP-5	267	383

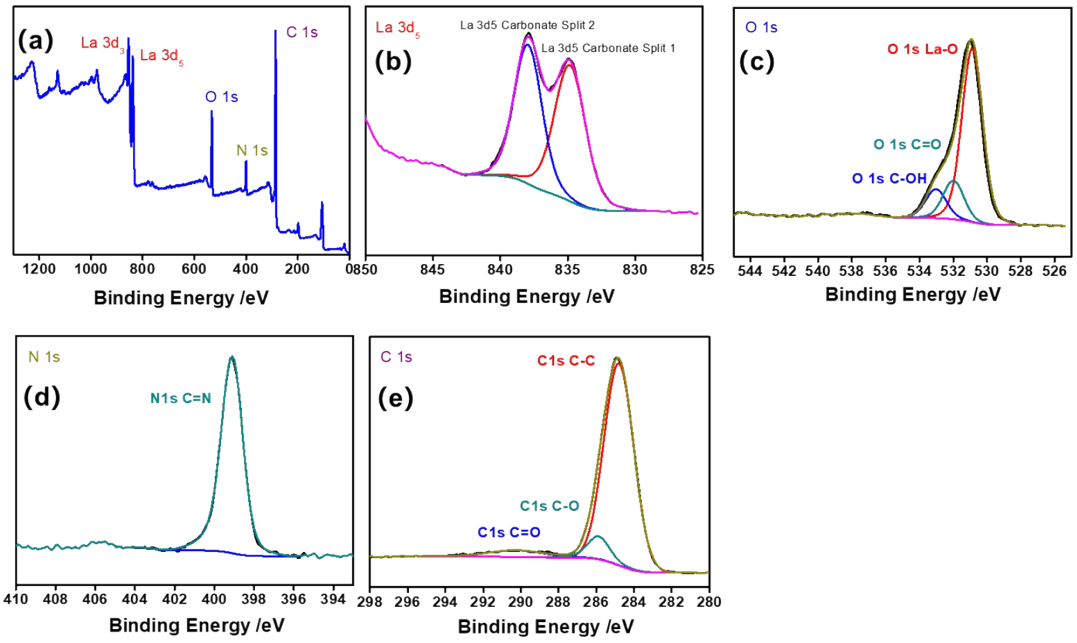

Figure S1 A. Survey spectra of GLCP, B. La 3d5 of GLCP, C. O 1s of GLCP, D. N1s of GLCP; E. C1s of GLCP

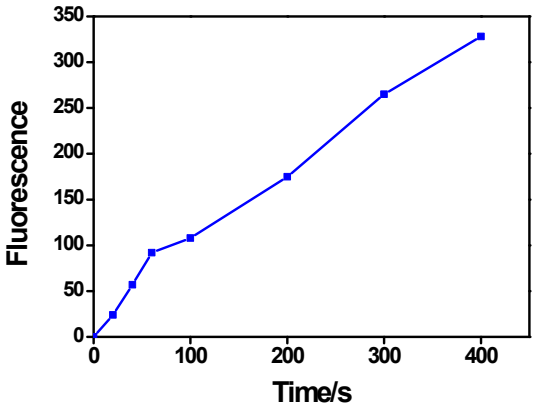

Figure S2. The ROS fluorescence signal of GLCP under visible light

Table S4. The bactericidal effect of the GLCP against different kinds of pathogenic bacteria

Bacteria	*E. coli*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*K. pneumonia*	*B. pumilus*	*B. subtilis*
Bactericidal effect（%	99.99	99.999	99.99	99	99.99	99.99	99.999

1. X. Yang, J. Qin, Y. Jiang, K. Chen, X. Yan, D. Zhang, R. Li and H. Tang, *Applied Catalysis B: Environmental*, 2015, **166-167**, 231-240.