Electronic Supplementary Information

Efficient Antibacterial activity via Protein Degradation of 3D Layered Double Hydroxide – Reduced Graphene Oxide Nanohybrid

EswaraVaraPrasadaraoKomarala, aSejalDoshi, a Aslam Mohammed b and Dhirendra Bahadura*

^aDepartment of Metallurgical Engineering and Materials Science, Indian Institute of Technology Bombay, Mumbai 400076, India.

^bDepartment of Physics, Indian Institute of Technology Bombay, Mumbai 400076, India.

*Corresponding author: Tel: +91 22 2576 7632, Fax: +91 22 2572 3480, E-mail: <u>dhirenb@iitb.ac.in</u>

Additional information about materials

Magnesium nitrate hexahydrate (Mg(NO₃)₂·6H₂O, 99%), aluminium nitrate nonahydrate (Al(NO₃)₃·9H₂O, 99%), ethylene glycol (C₂H₆O₂, 99%), sulphuric acid (H₂SO₄, 98%), hydrogen peroxide (H₂O₂, 30 wt%), diethyl ether (C₂H₅.O.C₂H₅, 99.5%), hydrochloric acid (HCl, 37%), potassium permanganate (KMnO₄, 99%), ammonia (NH₃, 30%) were obtained from Merck; urea (NH₂.CO.NH₂, 99.5%) and hydrazine hydrate (N₂H₄.H₂O, 80%) were obtained from Thomas Baker. Pure graphite powder (<45µm, 99.99%) and phosphoric acid (H₃PO₄, 99%) were purchased from Sigma-Aldrich. Ethanol (C₂H₅OH, 99.9%) was purchased from the Thermo Scientific Millipore system. A Nutrient broth medium was obtained from Himedia. All the materials were used as received without any further purification.

Materials characterization techniques

The morphological properties of the as-synthesized LDH-rGO nanohybrid were investigated using field emission gun transmission electron microscopy (FEG-TEM, JEOL JAM 2100F (200 kV)). X-ray diffraction (XRD) patterns of the LDH-rGO nanohybrid were recorded on a Philips powder diffractometer PW3040/60 with CuK α (1.5406Å) radiation. The X-ray photoelectron spectra (XPS) were obtained with PHI5000VersaProbeII using Al K α radiation (~ 1486.6 eV). Raman measurements were done using Witec alpha300 RAS with 532 nm laser source. Fourier transform infrared (FTIR) spectra were recorded on a JASCO spectrometer (6100 type-A) instrument in the range of 400 – 4000 cm⁻¹.The zeta potential values were obtained by ZetaPALS zeta potential analyzer (BI-200 Brookhaven Instruments).Further on, antimicrobial studies were done on the bacterial model, gram negative coccobacilli, *E. coli*.The cell interaction studies were investigated by FEG-Scanning electron microscopy (FEG-SEM, JSM-7600F (0.1 to 30 kV)).



Fig. S1 High resolution XPS spectra of LDH: (a) Mg2p, (b) Al2p; and LDH-rGO nanohybrid: (c) Mg2p, (d) Al2p.



Fig. S2 FTIR spectra of (a) GO, (b) LDH and (c) LDH-rGO nanohybrid



Fig. S3 XRD Pattern of reduced graphene oxide.

Reduced graphene oxide (rGO) was prepared by the same method as of nanohybrid without Mg and Al precursors and with 15 mmol of urea. The XRD pattern of rGO shows two broad peaks at 24.5° and 43.5° corresponds to (002) and (100) planes. An additional peak observed at 26° is likely from the traces formed due to the alignment of amine groups of urea on the rGO surface.¹



Fig. S4 Raman spectrum of reduced graphene oxide.

Fig. S4 shows the Raman spectrum of as-prepared rGO. The I_D/I_G ratio of rGO is about 1.02, which is higher than the graphene oxide (0.94). The increase in the ratio could also be attributed to the decrease in the average size of the sp² domains, which causes some unpaired defects caused by the removal of oxygen groups of GO during the urea hydrolysis.



Fig. S5 XPS survey spectrum of reduced graphene oxide.

The XPS spectrum of rGO shows a decrease in the intensity of O1s peak compared to the graphene oxide which indicates that most of the oxygen functional groups are reduced and hence the formation of reduced graphene oxide.



Fig. S6 TEM micrograph of reduced graphene oxide (rGO).



Fig. S7 Biocompatibility of LDH, rGO, and LDH-rGO nanohybrid on L929 cells.

The graphical representation in Fig. 5a confirms that LDH (2 mg/mL) is biocompatible, with a cell viability of $98.9\pm0.6\%$ after incubating with L929 cells for 24 h. On the contrary, a drop of around 15% in cell viability is observed when rGO (2 mg/mL) is treated with *E. coli*. It was observed that much difference was not attained in biocompatibility of LDH-rGO (94.8±0.2%) as compared to LDH. This observation confirms that LDH-rGO is biocompatible, and can be used further for any medical or human handling.

References

1. M. Kumar and S. Kumar, *RSC Advances*, 2015, **5**, 14871-14878.