Supporting Information

Fabrication of Non-toxic reduced Graphene Oxide Protein Nano-framework as Sustained Antimicrobial Coating for Biomedical Application

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Preparation of Intracellular Protein

The bacterial cultures were harvested from growth media by centrifugation at 12,000 rpm (14940 g) at 4 °C for 15 min and the obtained cell pellets were resuspended in ice cold 50 mM phosphate buffer (pH 7.0) and washed three times using same buffer to remove media. Cell pellets were then crushed using sea sand in a motor and pestle under cold condition. The obtained paste was collected by centrifugation at 12,000 rpm (14940 g) at 4 °C for 15 min in order to remove the cell debris. The supernatant containing intracellular proteins was collected and protein concentration in the extract was measured using Bicinchoninic acid (BCA) kit.[1] In case of fungi, the mycelia were harvested from growth media by filtration, washed with same phosphate buffer and dried using blotting paper. The blotted dried mycelia were cut into small pieces and then crushed using sea sand. The intracellular protein was collected by centrifugation at 12,000 rpm (14940 g) at 4 °C for 15 min and protein concentration was measured as described above.

Estimation of Intra and Extra Cellular Protein and Nucleic Acids

Freshly grown bacterial culture (10^6 CFU/mL) was treated with different concentration of rGO (0–80.0 μg/mL). The treated cells were centrifuged at 12,000 rpm (14940 g) for 15 min at 4 °C and supernatant containing extracellular proteins and nucleic acids was collected. The concentration of extracellular proteins and nucleic acids was then measured using Bicinchoninic acid (BCA) kit[1] and UV-vis spectrophotometer, respectively.

To analyze the intracellular proteins and nucleic acids, the cell pellets were dissolved in 1 mL of lysis buffer (50 mM Tris pH-8.0, 10% glycerol, 1% Triton X-100 and 1 mg/mL lysozyme) and incubate at 30 °C for 30 min under shaking (120 rpm). The cell suspension was then sonicated in an ice bath for 2 min (Q500 Sonicator). The cell lysate was collected by centrifugation at 12,000 rpm (14940 g) for 10 min. The protein and nucleic acids concentrations in cell lysate were measured using Bicinchoninic acid (BCA) kit[1] and UV-vis spectrophotometer, respectively.

Genomic DNA Isolation

The genomic DNA was isolated using the standard protocol.[2] In brief, 2 mL of the freshly grown bacterial culture (10^6 CFU/mL) was incubated with rGO (0–80.0 μg/mL) for 5 h at 30 °C. Cells were then collected by centrifugation 12,000 rpm (14940 g) for 10 min, washed with phosphate buffer (50 mM, pH 7.2) and resuspended in 890 μL of lysis buffer (50 mM Tris pH 8.0, 10 mM EDTA, 20% sucrose, 0.5% Triton X-100 and 100 μg/mL lysozyme). 100 μL of 10% SDS and 10 μL of Proteinase K (10 mg/mL) were added to the cells and further incubated at 37 ºC for 1 h. 1 mL of phenol-chloroform (1:1) mixture was then added into the cell suspension and incubated at 30 ºC for 5 min. After completion of incubation, the cell suspension was then centrifuged at 12,000 rpm for 10 min at 4 ºC. The highly viscous jelly like supernatant was collected in a fresh tube and the process was repeated with phenol-chloroform mixture. The sample was subsequently centrifuged at 12,000 rpm for10 min and supernatant was collected. 100 μL of 5 M sodium acetate solution was added into the supernatant and mixed gently by inverting the tube few times. After addition of 2 mL of isopropanol a white precipitation of DNA was collected by centrifugation at 5,000 rpm for 10 min. The DNA was subsequently collected and washed with 70% ethanol, which was then air dried for 5 min and suspended in 200 μL of TE buffer (50 mM Tris, pH 8.0 and 10 mM EDTA). The concentration of DNA was estimated using a spectrophotometer at 260/280 nm and analyzed by 1.5% agarose gel electrophoresis. The untreated culture was served as control and the DNA pattern of both treated and untreated cell was finally compared to measure the DNA damage due to rGO treatment.

Hemolysis Experiment

Fresh human blood was collected from healthy donor and and stabilized with EDTA. Red blood cells (RBC) were separated by centrifugation (12,500 rpm for 15 min), washed three times with sterile 50 mM phosphate buffer saline (PBS, pH 7.2), and diluted to a working concentration of 5% (v/v). Different concentration (20-80 μg/mL) of GO and rGO were added separately to 50 μL of RBC and incubated at 37 °C for 1 h with shaking (150 rpm). RBCs treated with 0.1% (v/v) Triton

X-100 was served as positive control, whereas, PBS was used as negative control. At the end of incubation, the solution was centrifuged (12,000 rpm for 15 min) and absorbance of the supernatant was measured at 540 nm using microplate reader (Tecan Infinite M200).

Cell Culture of 3T6 Fibroblast Cell Line

The 3T6 fibroblast cell line was purchased from National Centre for Cell Science (NCCS), Pune, India and cultured in DMSO medium supplemented with 10% (w/v) fetal bovine serum (FBS). Cells (10^5/well) were plated in 96 well plates containing 100 µL of medium/well and were grown to confluence at 37 °C in a humidity atmosphere containing 5% CO_2 in air.

Results

Table S1: Biomass Yield and Intracellular Protein Concentration of Different Species

Species	Biomass Yield (mg/mL)*	Protein (mg/mL)
P. sajor-caju	12.8 ± 1.35	2.78 ± 0.1
R. oryzae	9.1 ± 0.71	2.01 ± 0.16
S. oneidensis	5.4 ± 0.37	1.75 ± 0.39
S. algae	4.8 ± 0.41	1.56 ± 0.11
S. putrifaciens	5.1 ± 0.32	1.34 ± 0.08

*Dry biomass yield with respect to volume of growth media

Table S2: Intra and Extra-Cellular Protein and DNA Concentration of *E. coli* after Treatment with rGO Solution

rGO (µg/mL)	Intra-cellular substances			Extra-cellular substances		
	Protein (µg/mL)	DNA (µg/mL)	RNA (µg/mL)	Protein (µg/mL)	DNA (µg/mL)	RNA (µg/mL)
NC	1004.1 ± 18	26.2 ± 1.5	98.2 ± 9.1	97.3 ± 16.1	2.8 ± 0.2	37.1 ± 9.5
PC	462.3 ± 25.2	9.93 ± 1.6	55.5 ± 5.37	233.5 ± 41.6	15.6 ± 3.9	59.2 ± 8.2
20	904.2 ± 4.6	21.1 ± 2.5	92.6 ± 9.5	137.5 ± 25.7	4.8 ± 0.9	39.9 ± 7.6
40	811.1 ± 33.2	18.9 ± 6.2	88.13 ± 2.12	146.3 ± 3.65	5.34 ± 0.25	43.1 ± 9.7
60	718.5 ± 32.5	13.2 ± 2.3	70.95 ± 12.3	169.4 ± 14.6	6.4 ± 0.9	46.5 ± 8.9
80	655.5 ± 52.2	10.5 ± 7.1	65.4 ± 13.6	220.8 ± 17.5	11.9 ± 9.5	54.6 ± 12.6

Table S3: Intra and Extra-Cellular Protein and DNA Concentration of *E. coli* after Treatment with rGO Coated Glass

Glass substrate	Intra-cellular substances			Extra-cellular substances		
	Protein (µg/mL)	DNA (µg/mL)	RNA (µg/mL)	Protein (µg/mL)	DNA (µg/mL)	RNA (µg/mL)
Uncoated	232.0 ±3.1	33.3 ± 2.2	93.4 ± 3.2	93.1±2.2	1.6 ± 3.5	27.3 ± 5.4
rGO Coated	212.9 ± 1.5	28.6 ± 7.5	83.6 ± 1.6	118.6 ±1.3	2.5 ± 2.8	29.5 ± 6.5

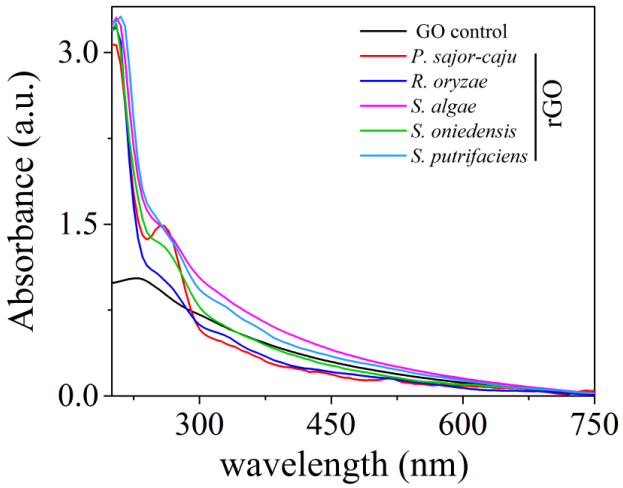

Figure S1. UV-vis spectrum for synthesis of rGO using biomass of different organisms.

Figure S2. Color image shows large scale (5 L) biosynthesis of rGO using intracellular protein of *P. sajor-caju* at ambient condition.

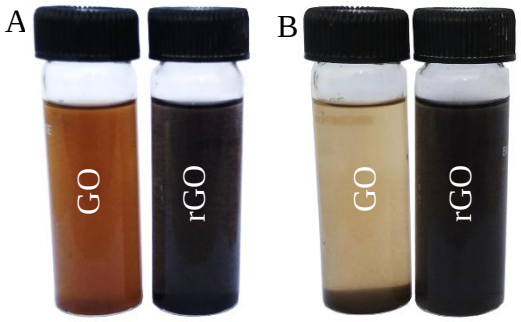

Figure S3. Color images of GO and rGO solution at 0^{th} min (A) and after 24 h (B) shows high dispersibility of rGO-protein nano-framework.

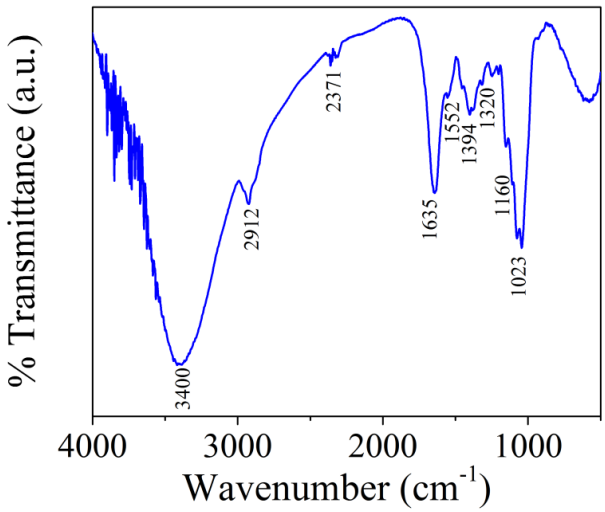

Figure S4. FTIR spectrum of cell free protein extract.

References

1. Huang, T.; Long, M.; Huo, B. Competitive Binding to Cuprous Ions of Protein and BCA in the Bicinchoninic Acid Protein Assay. *Open Biomed Eng J.* **2010**, *4*, 271-278.

2. Sambrook, J.; Russell, D.W. Molecular Cloning; a Laboratory Manual, 3rd ed. Cold Spring Harbor Press: New York, **2001**, Vol. 1.