# Mechanistic insight on the in vitro toxicity of graphene oxide against biofilm forming bacteria using laser-induced breakdown spectroscopy

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Supplementary Information Include:

5 Figures

2 Tables

## MTT assay protocol

Post ROS quantification, the cell viability was analyzed employing MTT dye (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Abcam, Hong Kong).[1] Cells uptake the MTT dye are enzymatically converted to insoluble formazan by NAD(P)H oxidoreductase enzyme intracellularly. This enzymatic catalysis will occur only when the cells are alive, and this will be helpful in determining the cell viability. After the supernatant was taken for ROS measurements the cell pellets were resuspended in PBS 10 µl PBS. Total reaction volume of 150 µl was maintained, containing 10 µl cells in PBS and 20 µl MTT dye. The final volume was made upto 150 µl with PBS (pH 7.2). The final mixture was further incubated for 4h at 37 °C, followed by addition of 100 µl DMSO to solubilize the formazan, which was spectrophotometrically (Spectramax M2e; Molecular Devices, Taiwan) recorded at 570 nm and compared with untreated controls cells to determine the percentage cell viability.

## Schematic of LIBS process

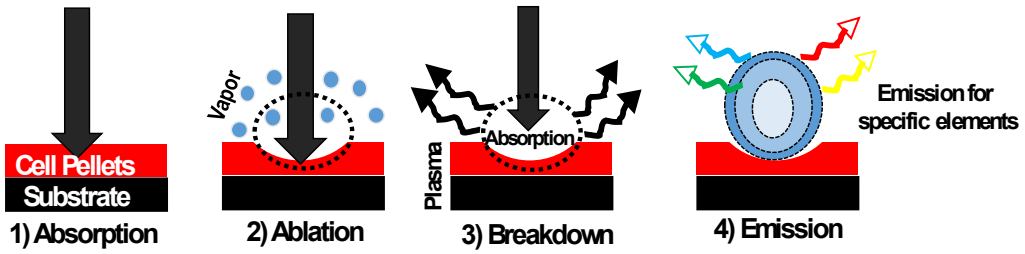


**Figure S1.** Schematic of LIBS process: 1. Absorption of energy by the sample from an incident laser pulse; 2. Absorbed energy will convert to heat, resulting in vaporization of sample (ablation) and the high-temperature plume expands outward; 3. The laser pulse continues to illuminate the vapor plume, later the heat leaks away through radiative heating and the plume cools and condenses back leading to absorption and scattering of the laser beam; 4. The resulting

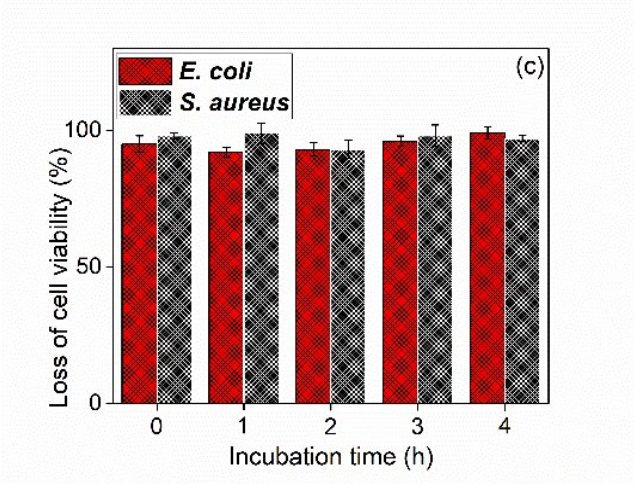


**Figure S2.** Loss of bacterial viability control cells.

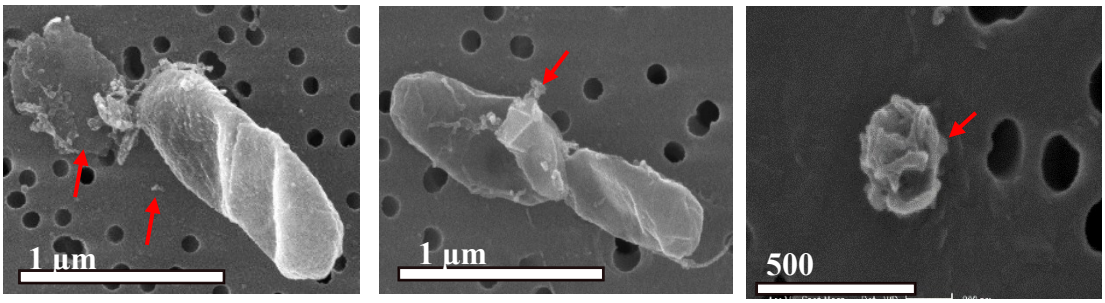


**Figure S3**. FE-SEM images of bacterial cells showing sever membrane disruption and efflux of intracellular substances

**Table S1**. Source and general physiological functions of major elements present in the bacterium.

| Elements | Dry Weight % | Source | Functions |
|---|---|---|---|
| Carbon | 50 | Organic compounds | Major constituents of cellular material |
| Oxygen | 20 | Organic compounds, $H_2O$, $CO_2$, and $O_2$ | Constituent of cell material and cell water; $O_2$ is electron acceptor in aerobic respiration |
| Nitrogen | 14 | Organic compounds $NH_3$, $NO_3$, and $N_2$ | Constituent of amino acids, nucleic acids nucleotides, and coenzymes |
| Hydrogen | 8 | Organic compounds, $H_2O$, and, $H_2$ | Main constituent of organic compounds and cell water |
| Phosphorous | 3 | Inorganic Phosphate ($PO_4$) | Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids |
| Sulphur | 1 | Organic sulfur compounds | Constituent of cysteine, methionine, glutathione, several coenzymes |
| Potassium | 1 | Potassium salts | Main cellular inorganic cation and cofactor for certain enzymes |
| Magnesium | 0.5 | Magnesium salts | Inorganic cellular cation, cofactor for certain enzymatic reactions |
| Calcium | 0.5 | Calcium Salts | Inorganic cellular cation, cofactor for certain enzymes and a component of endospores |
| Iron | 0.2 | Iron Salts | Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions |

**Data from:** (Kenneth, 2013)[2].

**Table S2:** Wavelengths corresponding to emission lines for individual elements in the LIBS spectra.

| Elements | Identification lines | Wavelength (nm) |
|---|---|---|
| Potassium | 1 | 766.5 |
| Oxygen | 1 | 777.2 |
| Carbon | 1 | 247.9 |
| Hydrogen | 1 | 656.3 |
| Magnesium | 2 | 279.6: 280.3 |
| Sodium | 2 | 589: 589.6 |
| Phosphorous | 3 | 213.6: 214.9: 253.6 |
| Calcium | 3 | 393.4: 396.8: 422.7 |

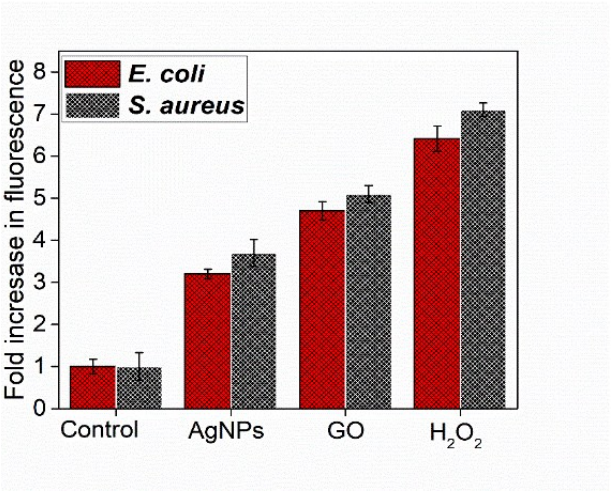


**Figure S4**. (a) Intracellular ROS generation in E. coli and S. aureus cells

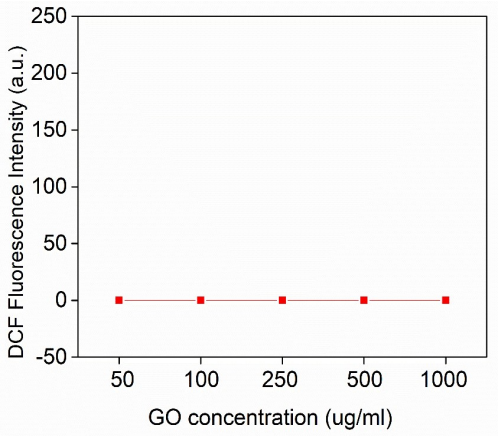


**Figure S5**. Acellular ROS generation at different GO concentrations.

**References**


1. Zhang, X., Hu, W., Li, J., Tao, L. and Wei, Y., 2012. A comparative study of cellular uptake and cytotoxicity of multi-walled carbon nanotubes, graphene oxide, and nanodiamond. Toxicology Research, 1(1), pp.62-68.
2. Kenneth, T. Todar's Online Textbook of Bacteriology. "The Good, the Bad, and the Deadly". 2013.