How graphene family materials affect *Listeria monocytogenes* and *Salmonella enterica* strains

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Introduction

The study compared the toxicity of different forms of graphene family materials (GFM); **pristine graphene (pG), graphene oxide (GO) and reduced graphene oxide (rGO) towards bacteria strains.**

The effect of three different GFMs on chosen food-borne bacteria strains: Gram-positive (G+) – *Listeria monocytogenes*, and Gram-negative (G-) – *Salmonella enterica* were examined.

Methods

The visualization of GFM and their interactions with bacteria were inspected using a JEM-1220 (JEOL, Tokyo, Japan) transmission electron microscope (TEM) at 80 KeV, with a Morada 11-megapixel camera (Olympus Soft Imaging Solutions, Münster, Germany).

Bacteria strains (5 x 10^8 cfu/ml suspended in 0,85% NaCl) were mixed with solution of pG, GO and rGO (25 mg/L and 250 mg/L) in ultra pure water, gently mixed and incubated overnight at room temperature.

Results

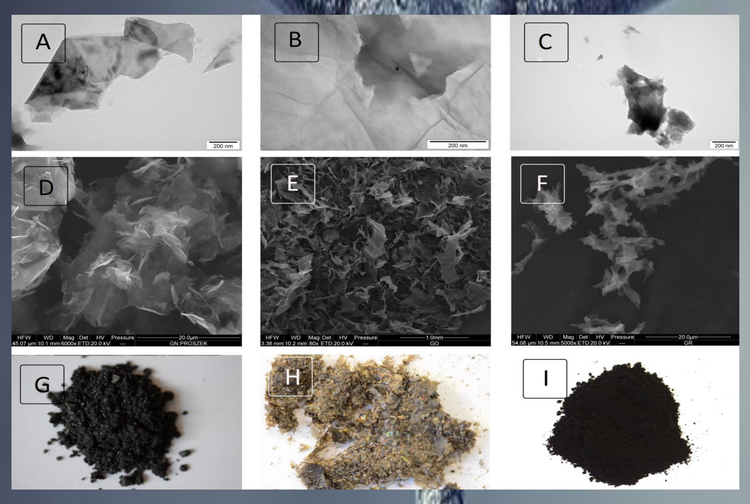

Figure 1. GFM were visualized using transmission electron microscopy (A-C), scanning electron microscopy (D-F), and a digital camera (G-I). Images of pristine graphene **(A, D, G)**, graphene oxide **(B, E, H)**, and reduced graphene oxide **(C, F, I)**.

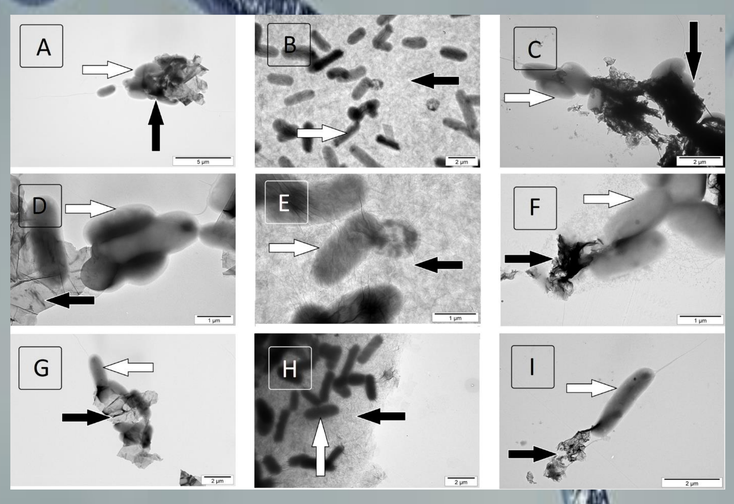

Figure 2. Visualization of the interaction of graphene family materials with*Salmonella enterica*using transmission electron microscopy. Pristine graphene **(A, D, G)**, graphene oxide **(B, E, H)**, and reduced graphene oxide **(C, F, I)**. Black arrows indicate the graphene material and white arrows the bacterial cells.

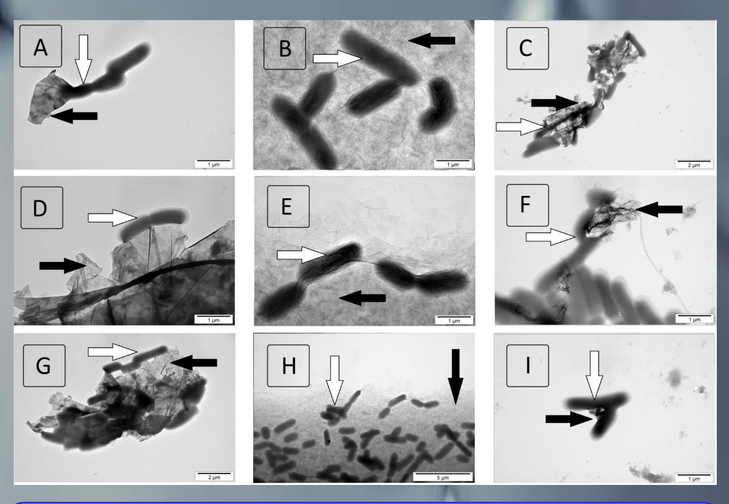

Figure 3. Visualization of the interaction of graphene family materials with*Listeria monocytogenes*using transmission electron microscopy. Pristine graphene **(A, D, G)**, graphene oxide (**B, E, H)**, and reduced graphene oxide **(C, F, I)**. Black arrows indicate the graphene material and white arrows the bacterial cells.

	pG	GO	rGO
Shape	Irregular, angular, single to a few layers	Film-like, rounded, single layers	Irregular, frayed, a few layers
Average size µm	1,86	1,27	2,53
Potential Zeta mV	-19,9	-49,8	-25,1
Surface chemical bonds evidence by FTIR spectra	C=C,	□-H, C=C, C=O, C-O, C-H	C=O, C=C, C-O, C-H
Hydrophilic	Not good	Good	Not good

Table 1. Physical and chemical characterization of pristine graphene (pG), graphene oxide (GO) and reduced graphene oxide (rGO).

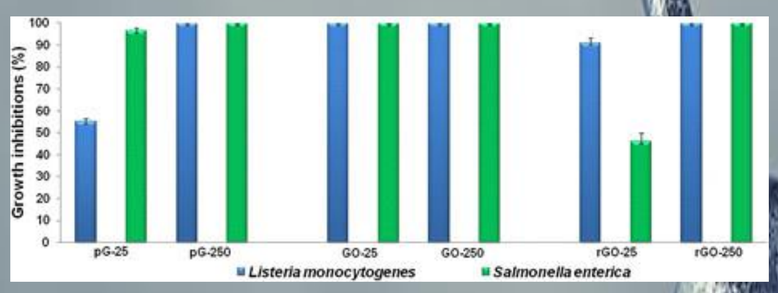

Figure 4. Influence of pG, GO, and rGO on the growth of *Listeria monocytogenes* and *Salmonella enterica*at 25 and 250 µg/mL. Data presented are the average of triplicate determinations, with error bars representing mean standard error.

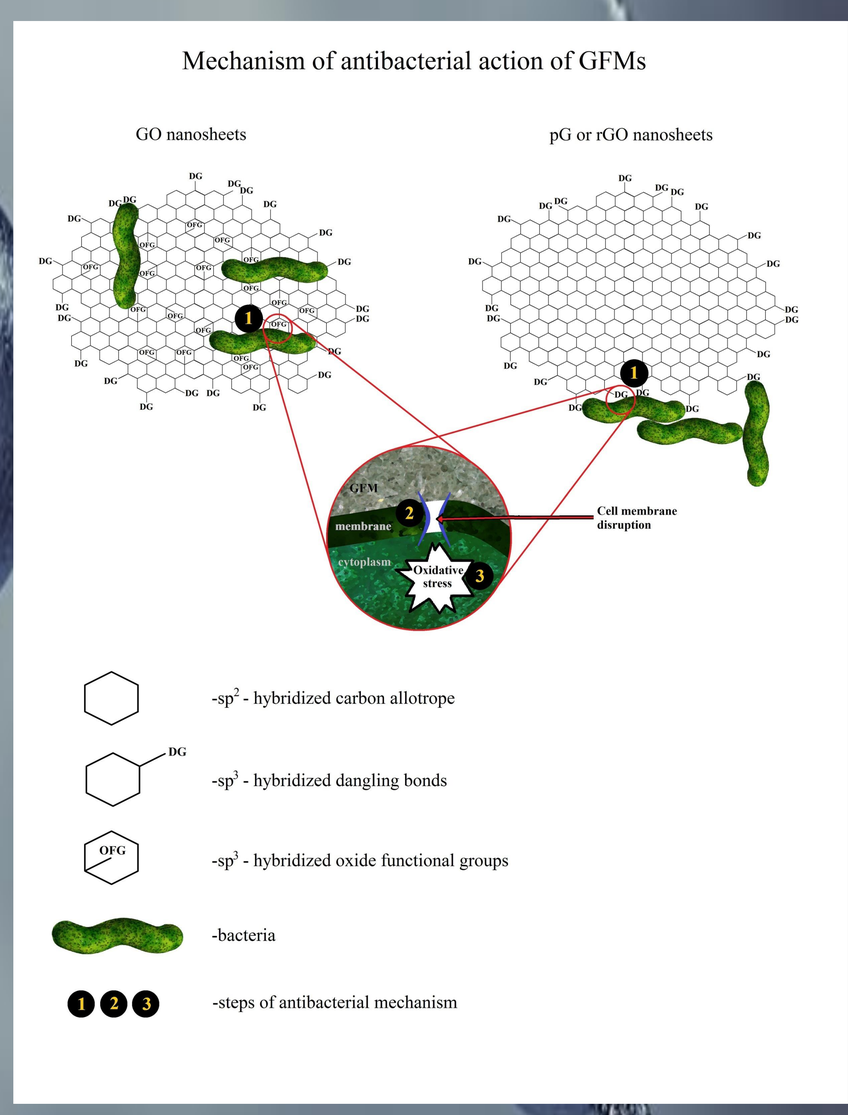

Figure 5. Three-step antimicrobial mechanism of graphene materials. 1. Initial bacteria cell deposition on graphene materials. 2. Membrane stress caused by direct contact with sharp edges. 3. Oxidative stress in the bacterial cytoplasm. The key difference between the chosen graphene materials is the bacterial cell deposition place. Individual bacterial cells interact with the sp^3-hybridized oxidative functional group of the GO surface, while bacterial cells interact with the sharp edges of pG and rGO and form a rope-like structure.

Conclusions

Results are a decreased number of bacteria colonies were observed in probes with 250 µg/mL for all examined GFMs. Moreover, as low concentration of GO as 25 µg/mL caused a drop in the level of bacterias colonies as well and reduced growth by almost 100%.

Bacteria were aggregated and attached to GFMs. A strong affinity occurred between bacteria and edges of pG and rGO, while bacteria strains attached to GO nanoparticle surfaces.

The present results indicate that GFM antibacterial activity causes mechanical damage of bacterial cell membranes by a direct contact of the bacteria with the extremely sharp edges of GFM with sp3-hybridized bonds. Based on the present results, we propose a three-step antimicrobial mechanism of GFM. It includes initial cell deposition on GFM (step 1), membrane stress and disruption caused by direct contact with sharp edges and bonds (step 2), and finally stimulated oxidation stress (step 3). The key difference between the chosen graphene materials is the bacterial cell deposition place.