

Supporting Information

Graphene Oxide Coated Surface: Inhibition of Bacterial Biofilm Formation due to Specific Surface-Interface Interactions

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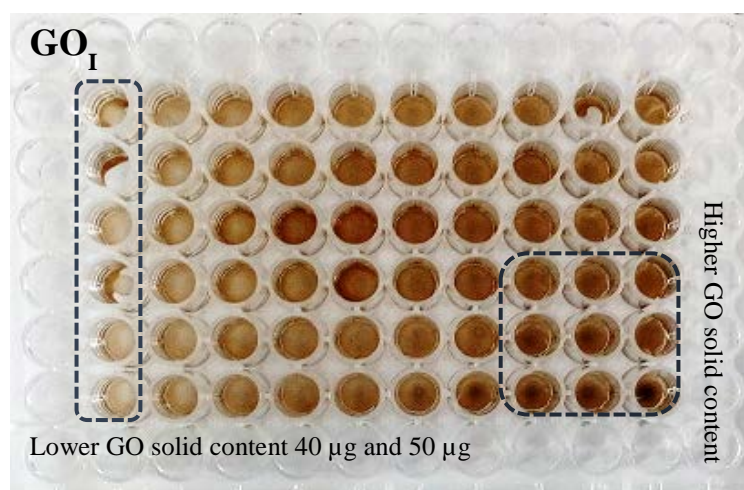
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Increasing GO content



Increasing GO content

Figure S1. Digital images of GO coated well plates of different GO solid content loading.

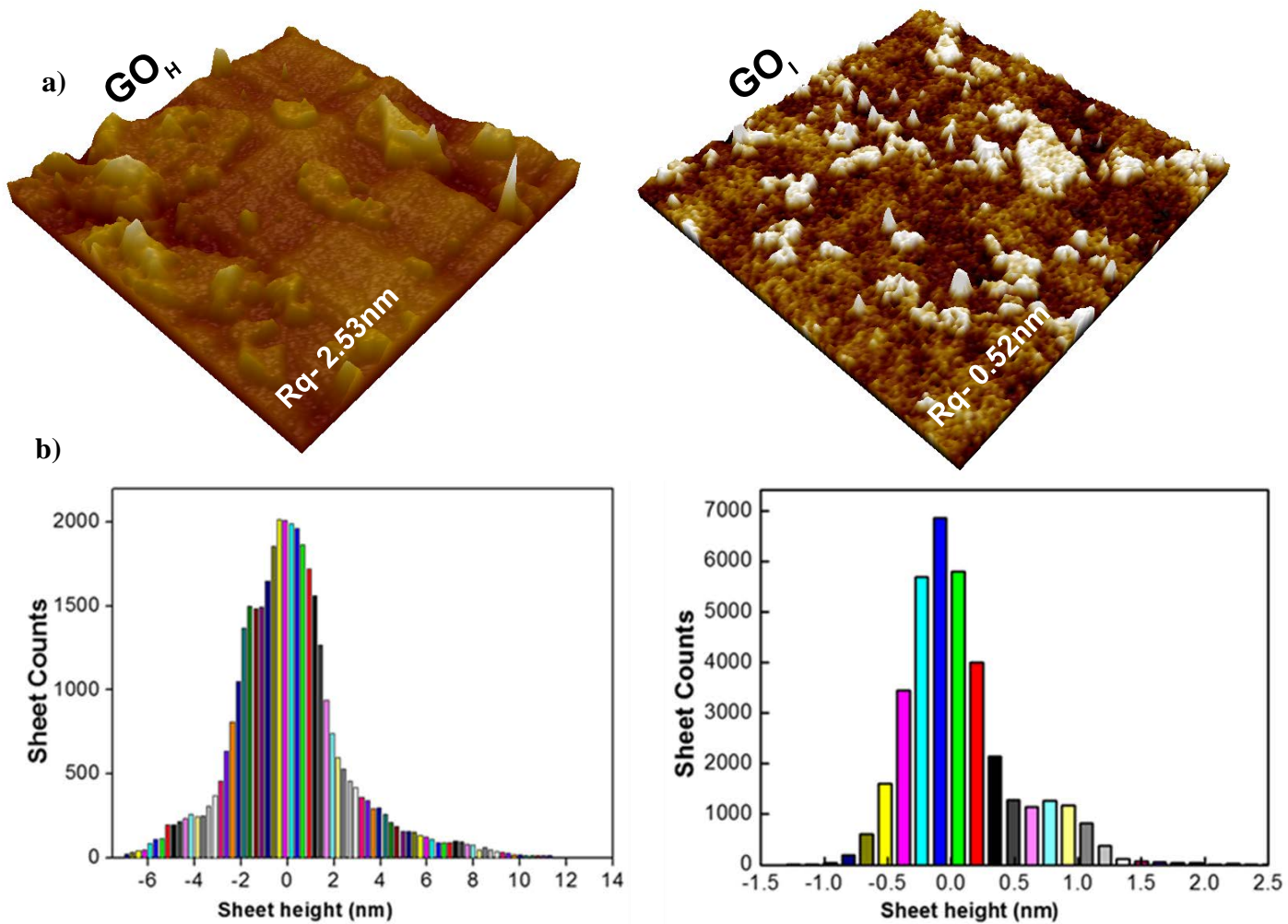


Figure S2. AFM imaging Graphene Oxide (GO_H and GO_L) deposited on a Si substrate and corresponding Rq values (a) 3D-AFM images (b) Sheet height distribution.

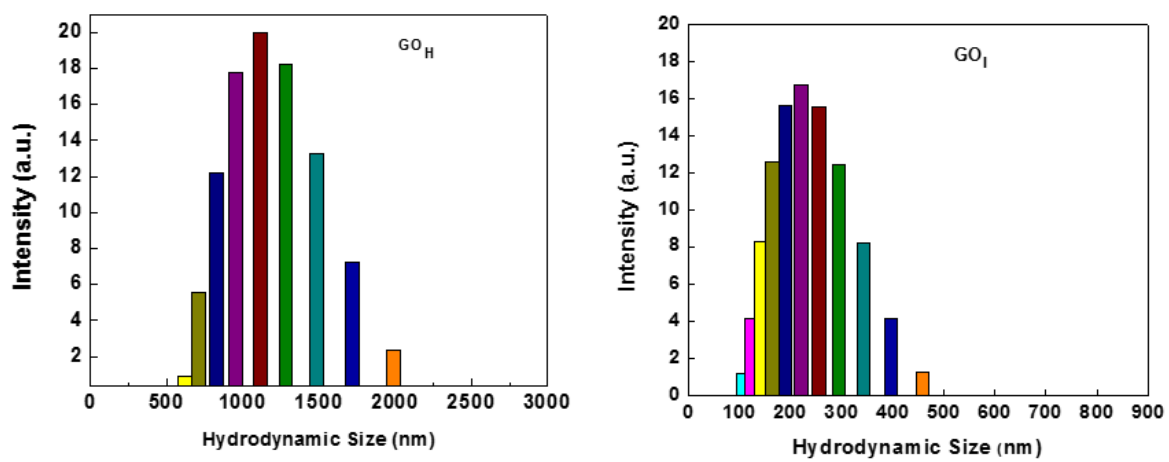


Figure S3. Hydrodynamic mean diameter and size distribution of GO_H and GO_I .

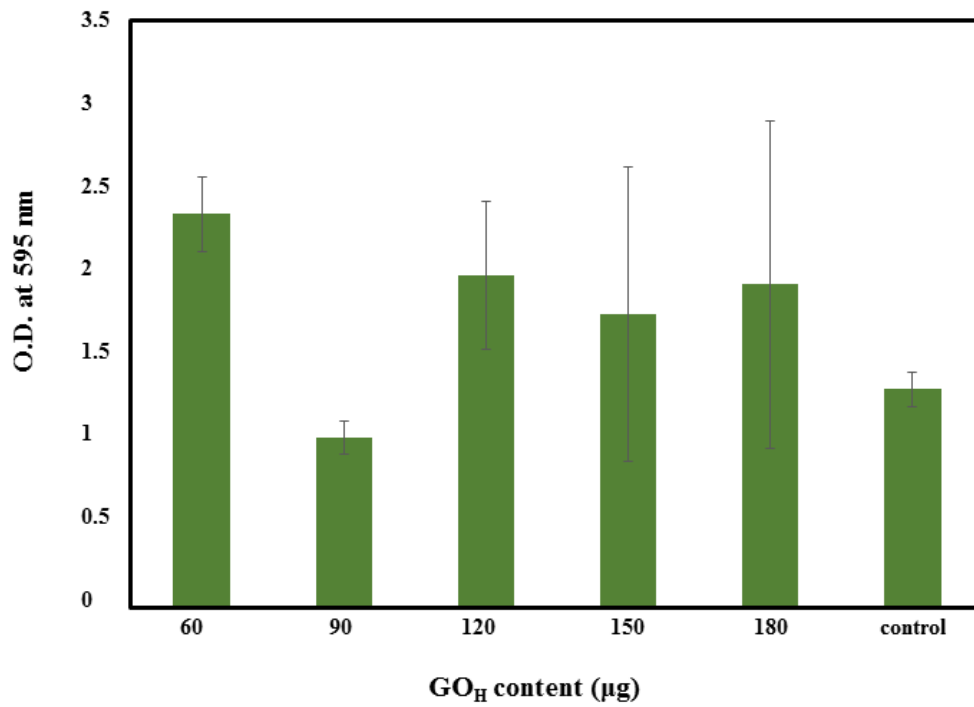


Figure S4. Quantitative analysis of antibacterial activity of GO_H coated surface against *E. coli* bacterial biofilm formation. Graphical representation of percentage biofilm inhibition in *E. coli*. Data represented here shows statistical difference (p-value < 0.05) between the GO_H treated and control sample.

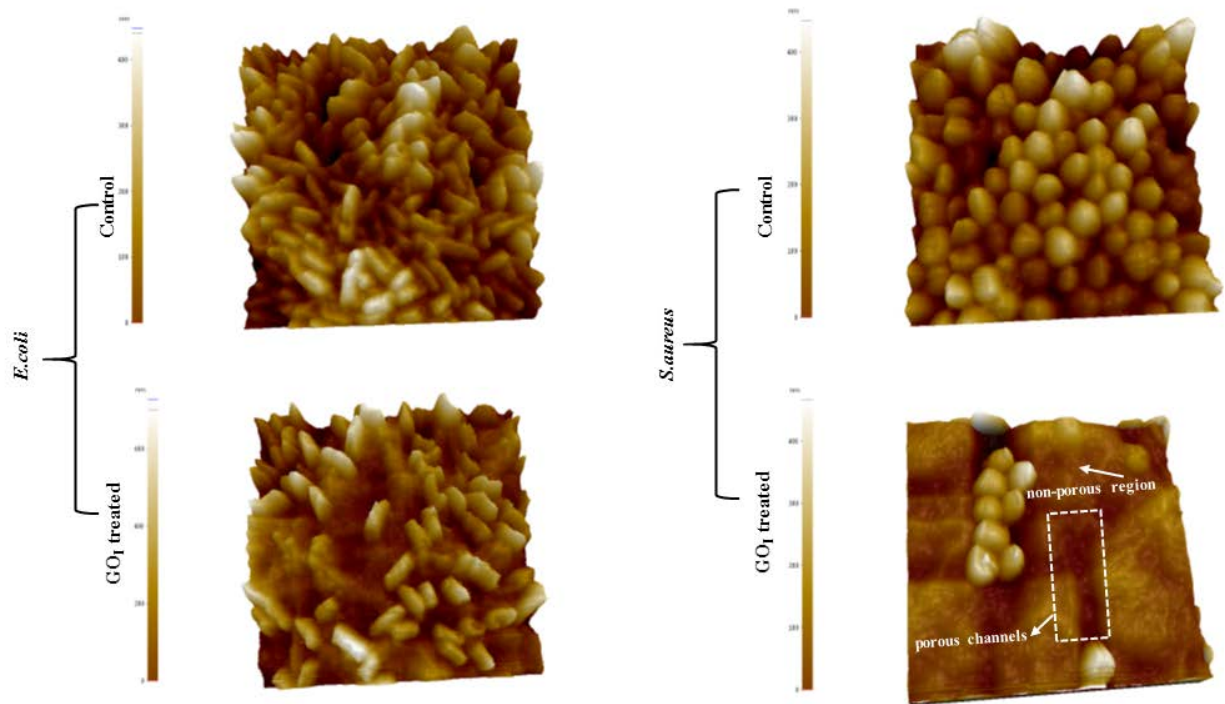


Figure S5. 3D AFM images of *E. coli* and *S. aureus* cells after incubation with GO₁ coated substrate. The control without GO nanosheets showed a dense biofilm formation, while GO₁ coated substrate showed a few cell colony of bacteria formation within the porous channels of GO-coated substrate and localized within the channels.

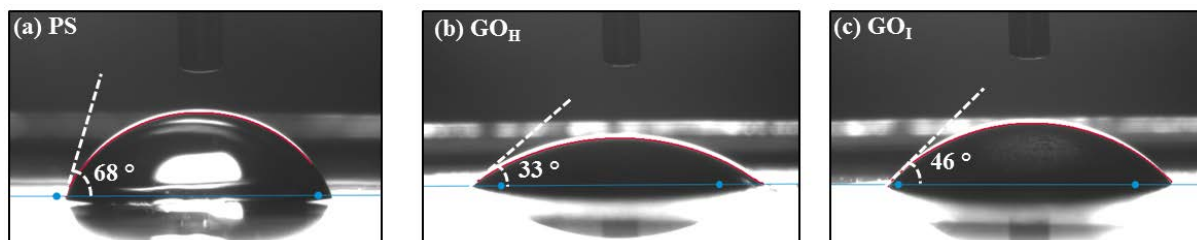


Figure S6. Images of contact angle measurements using MilliQ water in contact with (a) polystyrene (PS) substrate (control), (b) GO_H coated on PS and (c) GO_I coated on PS.

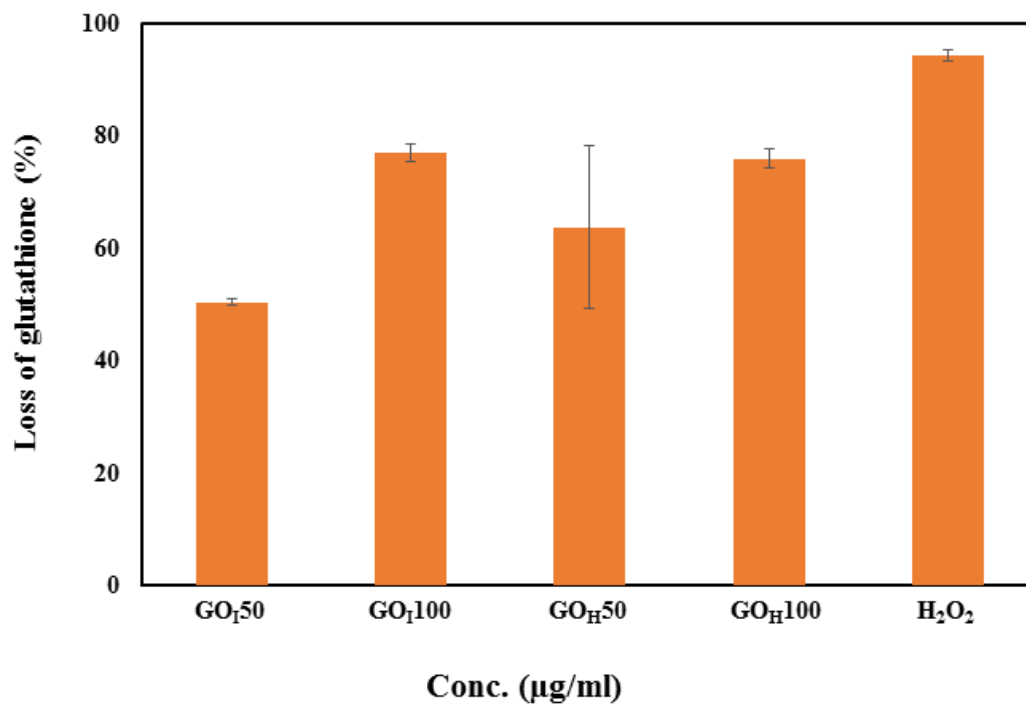


Figure S7. *In vitro* glutathione oxidation induced by GO nanomaterials: 50 µg/mL and 100 µg/mL of GO_H and GO_I were incubated with 0.8 mM glutathione under for 3 h. H₂O₂ (1 mM) represents positive control.

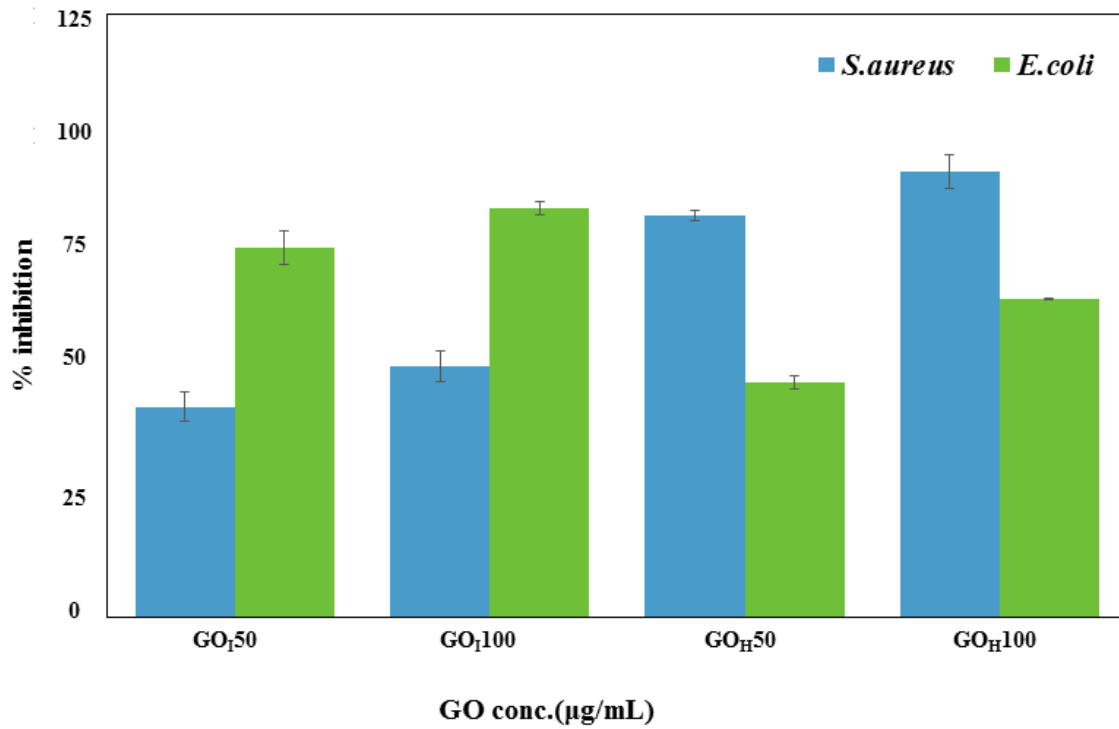


Figure S8. Antimicrobial activity of GO_H and GO_I. *E. coli* and *S. aureus* cells were incubated with different concentration (50 μg/mL and 100 μg/mL) of GO_H and GO_I. Error bars in the figure represents standard deviation.

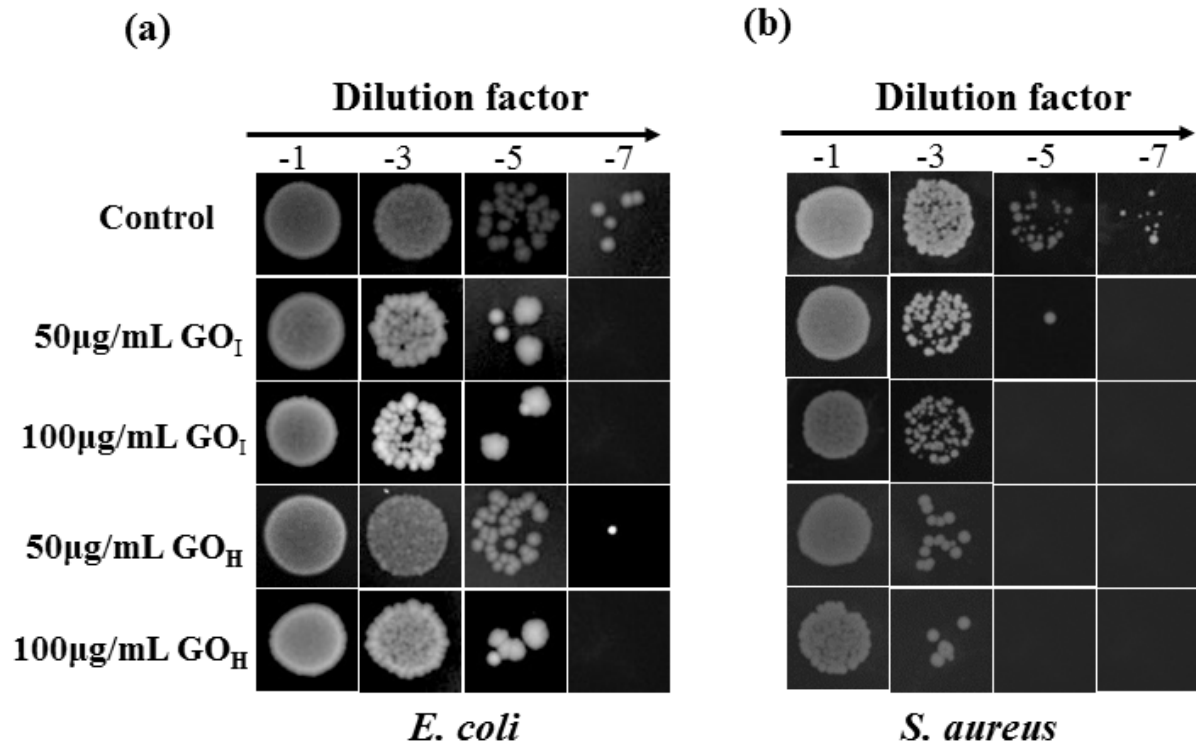


Figure S9. Spot assay representing antimicrobial activity of GO_I and GO_H at 50 µg/mL and 100 µg/mL (a) *E. coli* and (b) *S. aureus*.

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| GO solid content (μg) | GO (2 mg/mL) (μl) | H₂O (μl) |
|--|--|--|
| 60 | 30 | 220 |
| 90 | 45 | 205 |
| 120 | 60 | 190 |
| 150 | 75 | 175 |
| 180 | 90 | 160 |
| 200 | 100 | 150 |

Table S1: A series of different GO solid content (ranging from 60 μg to 200 μg) was prepared from the stock aqueous suspension of respective GO (2 mg/mL) onto 96-well plate. The volume of solvent was kept constant (250 μL) in each well of the plate to allow similar extent of drying in an air-oven at 50 °C for 4 h.

| Sample | GO solid content (μg) | Thickness_{at edge} (nm) | Thickness_{in middle} (nm) | Difference (nm) |
|-----------------|--|---|---|----------------------------|
| GO _H | 60 | 624 | 742 | 118 |
| | 200 | 825 | 1161 | 336 |
| GO _I | 60 | 51 | 88 | 37 |
| | 200 | 321 | 351 | 30 |

Table S2: Surface thickness measurement of GO_H and GO_I coated surface using surface profilometer. The results reported are the average of three measurements in different directions.

| Sample | XRD | | XPS (% Component) | | | |
|-----------------|------------|-------|-----------------------------|------------------------------|--------------------------------|----------------------|
| | 2 θ | d (Å) | C-C, C=C, C-H (285.0 eV) | C-OH, Epoxy (286.2 eV) | C=O, Carbonyl (288.1 eV) | O=C-OH (289.6 eV) |
| GO _H | 11.25 | 7.8 | 6.8 | 41.4 | 39.9 | 11.9 |
| GO _I | 9.8 | 9.04 | 18.7 | 53.3 | 23.6 | 4.4 |

Table S3: XRD data (d – Interlayer spacing) and XPS deconvoluted percentage component of GO_H and GO_I.

| Study | Synthetic Method for GO preparation | Bacterial Strain | Concentration/sonication time | Inhibition (%) | Ref. |
|---|-------------------------------------|--|--|--|-----------|
| GO coated surface for inhibition of Biofilm formation | Improved Hummers | <i>E. coli</i> <i>S. aureus</i> | >150µg GO solid content | ≥ 100 in <i>E.coli</i> 88% in <i>S. aureus</i> (in Biofilm inhibition) | This work |
| Suspension | Improved | <i>E. coli</i> | 100 µg/mL /2 min. | 85 | This work |
| | Hummers | <i>S. aureus</i> | 100 µg/mL /2 min. | 93 | |
| Suspension | Modified Hummers and Offeman | <i>E. coli</i> | 40 µg/mL /n.a. | 69.3 | 1 |
| Suspension | Modified Hummers and Offeman | <i>E.coli</i> | 40 µg/mL /0-240 | 97.7-45.5 | 2 |
| Suspension and coated cellulose filter | Modified Hummers and Offeman | <i>E.coli</i> | 400 µg solid GO content for coated and 200 µg for Suspension /0-120 | 79-21 for coated 70-27 for Suspension | 3 |
| Highly wrinkled GO films | Modified Hummers and Offeman | <i>E. coli</i> , <i>S. aureus</i> , <i>Mycobacterium</i> | Surface roughness Rq-500nm >1000µg/mL | 80 | 4 |

Table S4: Comparison of our GO coated substrate with previously reported literature in terms of percentage inhibition at the respective concentration.

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References

- 1) Liu, S.; Zeng, T.H.; Hofmann, M.; Burcombe, E.; Wei, J.; Jiang, R.; Kong, J.; Chen, Y. Antibacterial Activity of Graphite, Graphite Oxide, Graphene Oxide, and Reduced Graphene Oxide: Membrane and Oxidative Stress. *ACS Nano*, **2011**, *5*, 6971-6980.
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