

Enhanced Antibacterial Activity through Silver Nanoparticles Deposited onto Carboxylated Graphene Oxide Surface

Arturo Barjola ¹, María Ángeles Tormo-Mas ^{2,*}, Oscar Sahuquillo ¹, Patricia Bernabé-Quispe ², José Manuel Pérez ² and Enrique Giménez ^{1,*}

¹ Instituto de Tecnología de Materiales, Universitat Politècnica de València (UPV), Camino de Vera s/n, 46022 Valencia, Spain; arbarrui@doctor.upv.es (A.B.); ossana@upvnet.upv.es (O.S.)

² Severe Infection Group, Health Research Institute La Fe, University and Polytechnic La Fe Hospital, Avda. Fernando Abril Martorell 106, 46026 Valencia, Spain; patricia_bernabe@iislafe.es (P.B.); manuel_perez@iislafe.es (J.M.P.)

* Correspondence: tormo_man@iislafe.es (M.Á.T.-M.); enrique.gimenez@mcm.upv.es (E.G.)

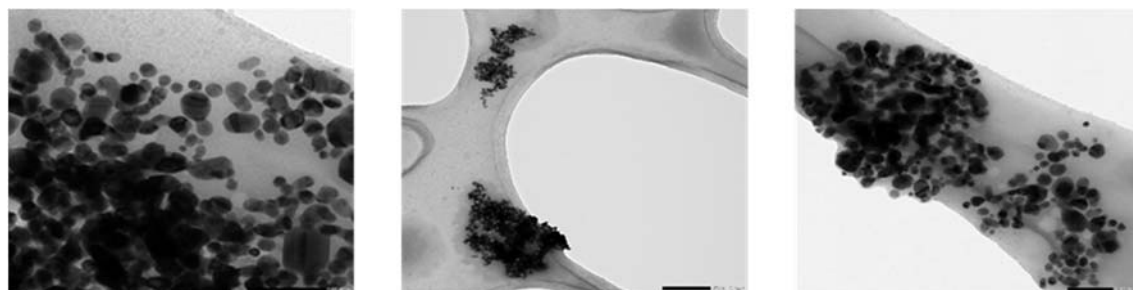


Figure S1. TEM micrographs of AgNPs.

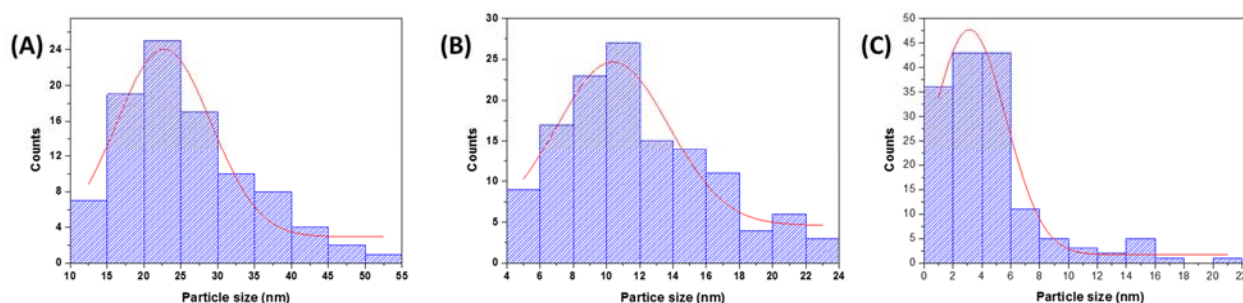


Figure S2. Histograms and Gaussian fit for the particle size of (A) AgNPs, (B) GO-Ag, and (C) GOCOOH-Ag silver nanoparticles.

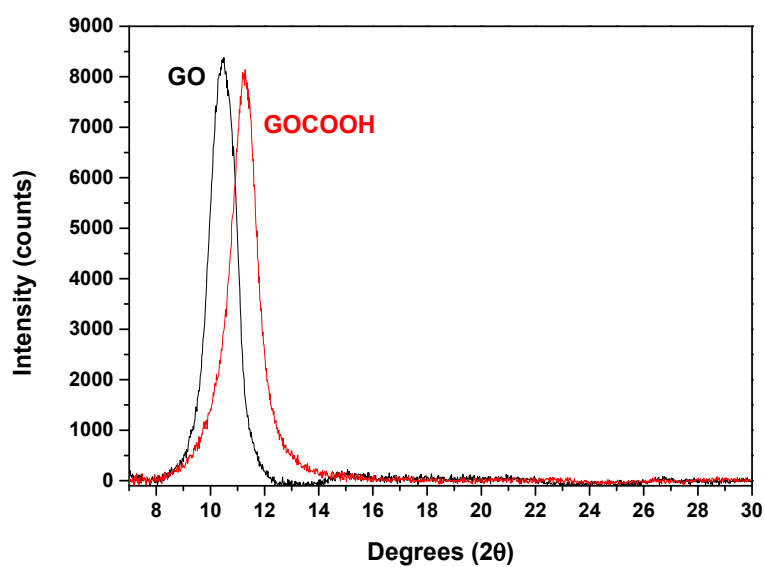


Figure S3. XRD spectrum for GO (black) and GOCOOH (red).

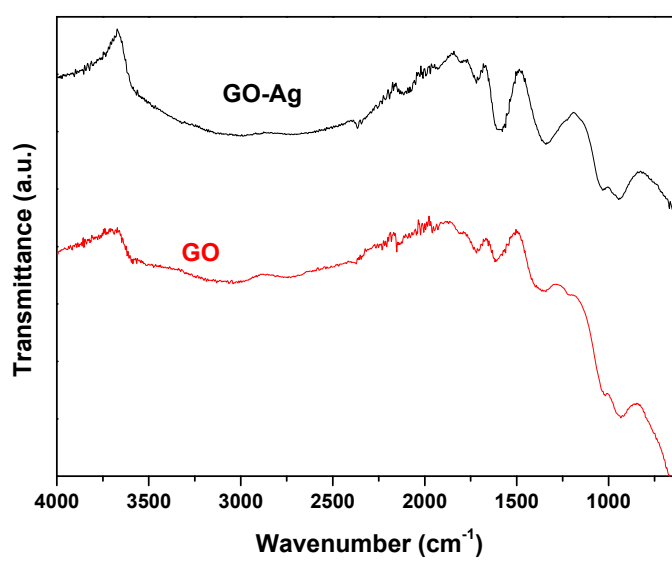


Figure S4. FTIR spectrum GO (red) and GO-Ag (black).

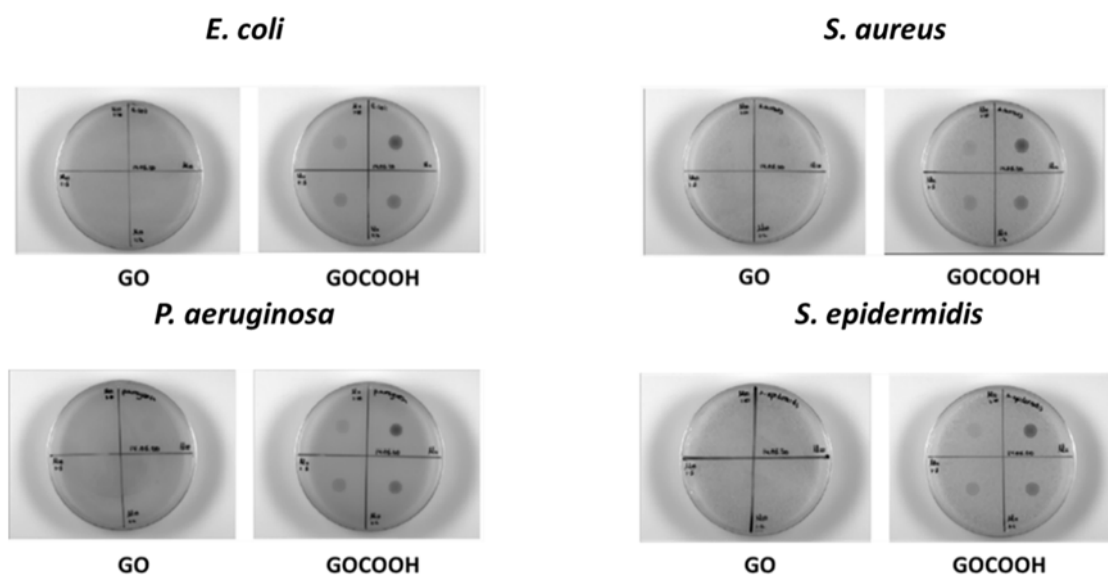


Figure S5. Kirby-Bauer plate diffusion method applied for GO and GOCOOH initial screening.

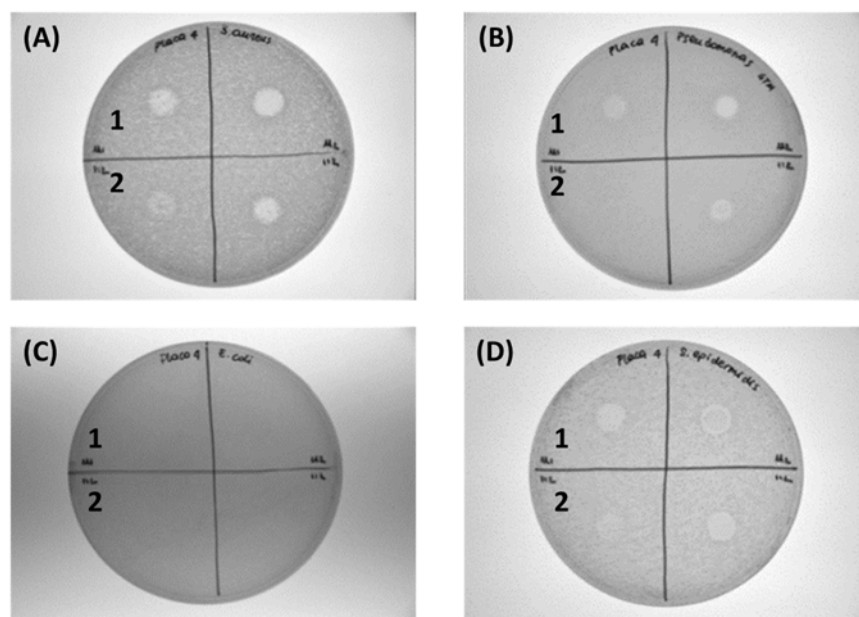


Figure S6. Kirby-Bauer plate diffusion method applied for AgNP initial screening. (A) *S. aureus*, (B) *P. aeruginosa*, (C) *E. coli*, and (D) *S. epidermidis*. AgNP concentration was 25 µg/mL for (A1, B1, C1, D1) and 12.5 µg/mL for (A2, B2, C2, D2).

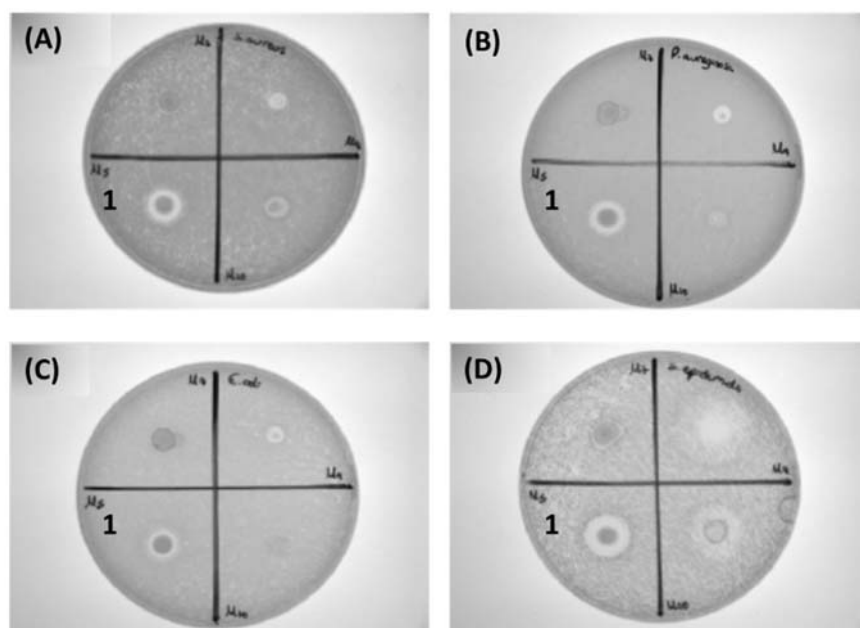


Figure S7. Kirby–Bauer plate diffusion method applied for GO-Ag initial screening. (A1) *S. aureus*, (B1) *P. aeruginosa*, (C1) *E. coli*, and (D1) *S. epidermidis*. AgNP concentration was 12.5 $\mu\text{g/mL}$.

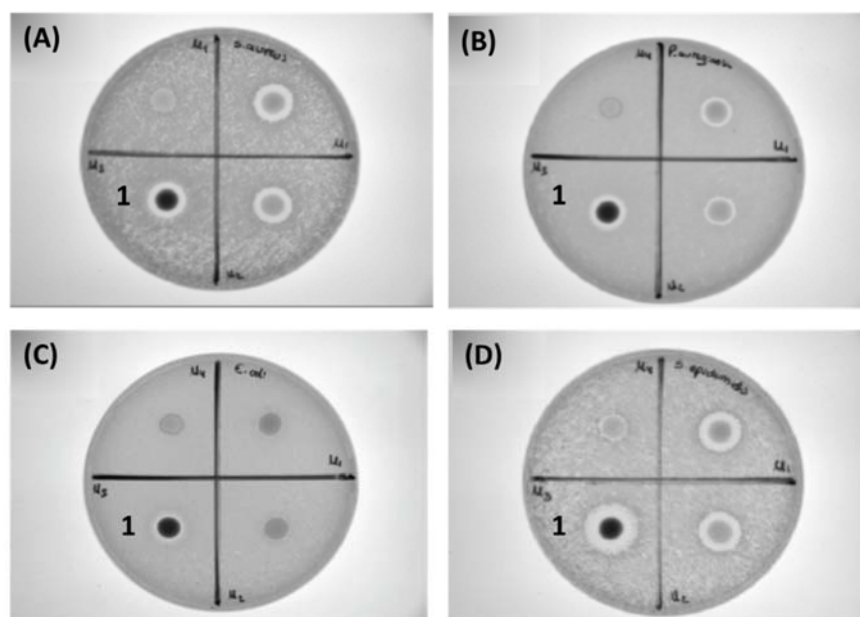


Figure S8. Kirby–Bauer plate diffusion method applied for GOCOOH-Ag initial screening. (A1) *S. aureus*, (B1) *P. aeruginosa*, (C1) *E. coli*, and (D1) *S. epidermidis*. AgNP concentration was 12.5 $\mu\text{g/mL}$.

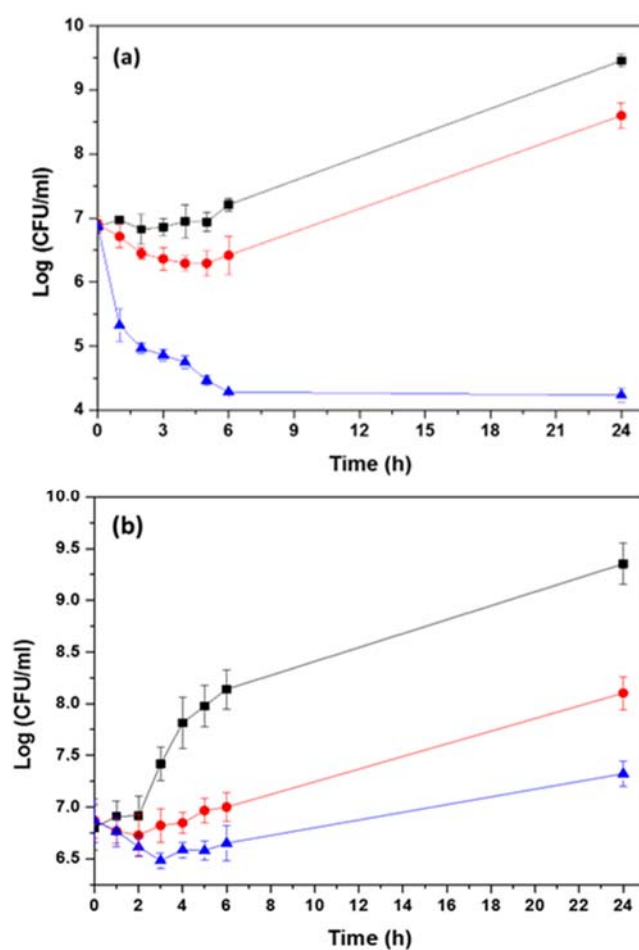


Figure S9. Growth curves of (a) *S. aureus* ATCC25423 and (b) *S. epidermidis* ATCC32984 in the presence of GO-Ag (red circles) and GOCOOH-Ag (blue triangles) at 12.66 $\mu\text{g/mL}$. Control is depicted with black squares. The data were presented as means and SD of at least three independent experiments.

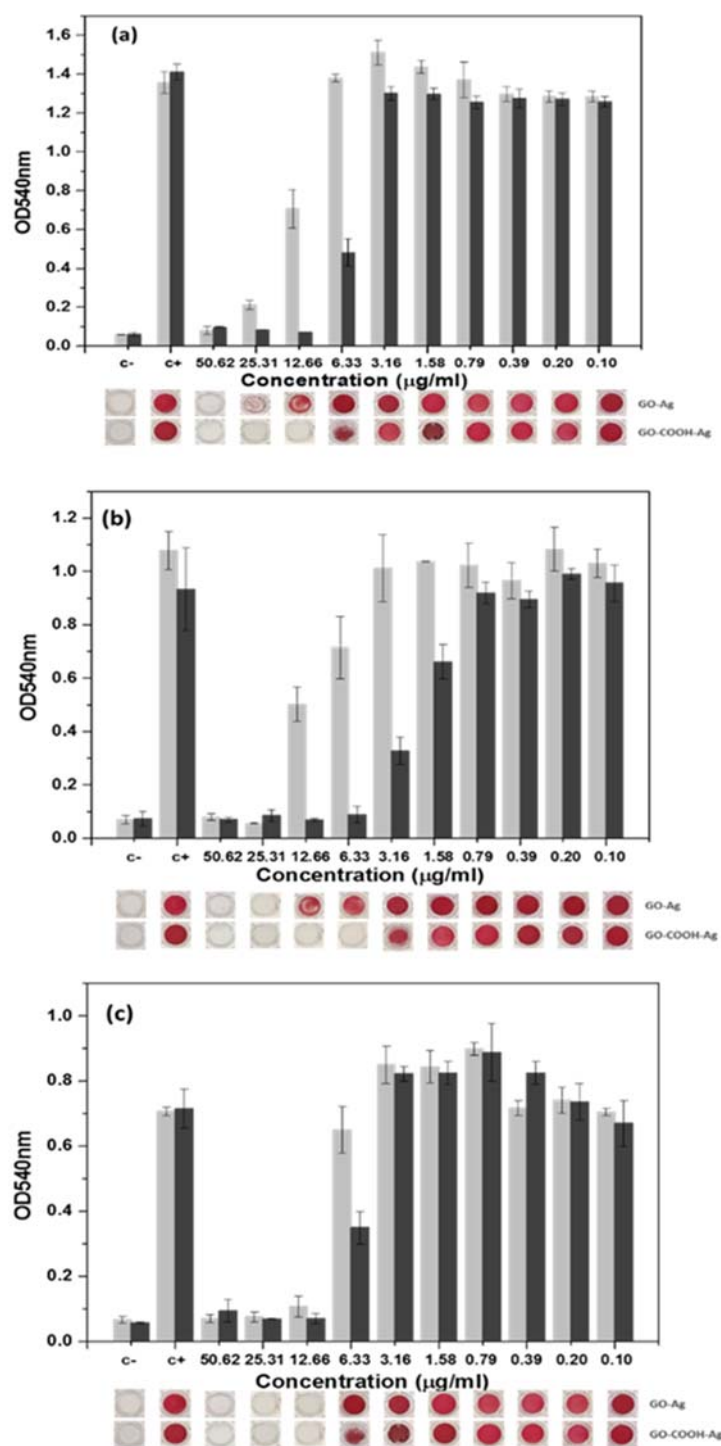
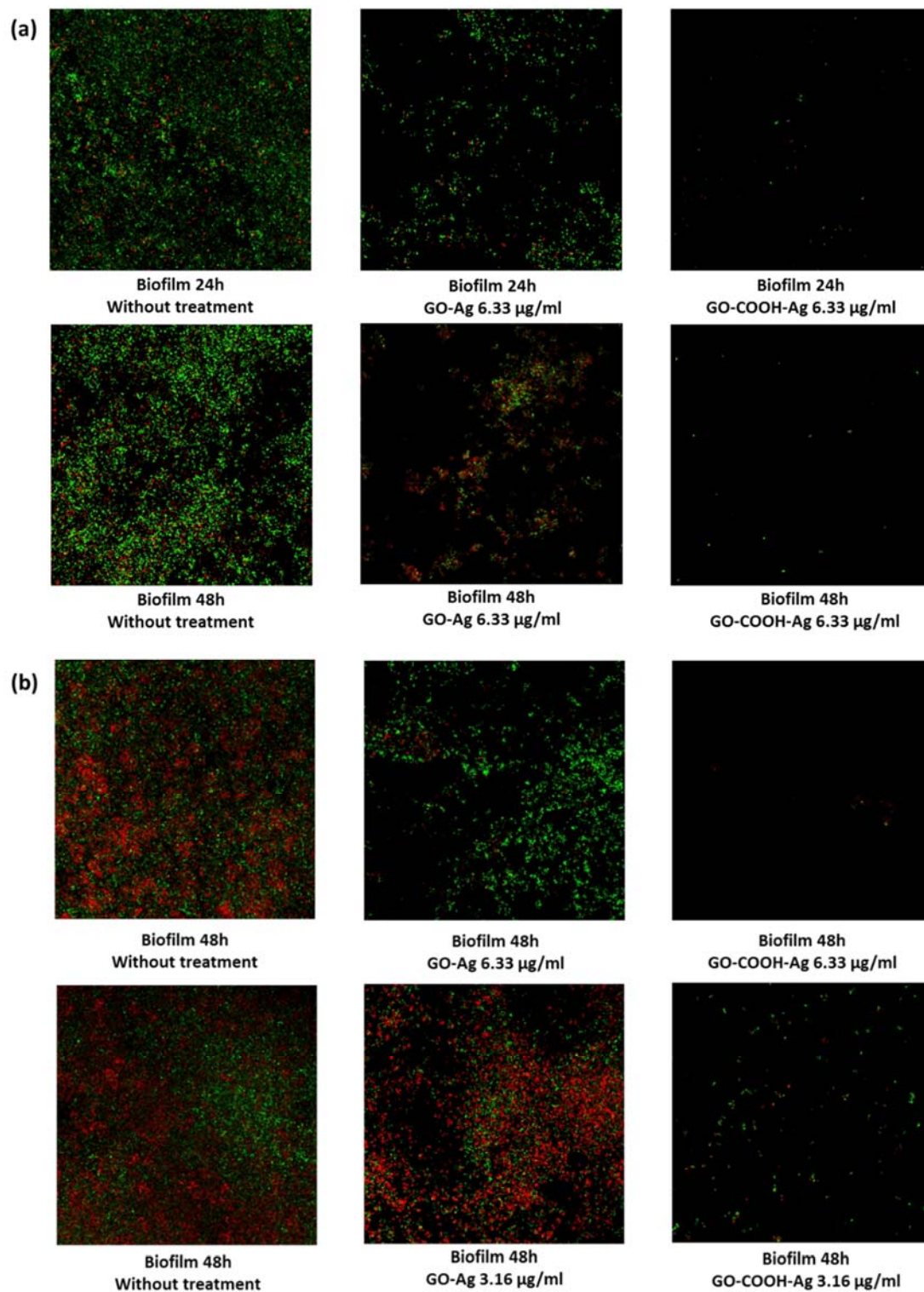


Figure S10. Effect of GO-Ag (light grey) and GOCOOH-Ag (dark grey) hybrid nanomaterials on the biofilm of (a) *S. epidermidis* RP62A; (b) *P. aeruginosa* PFQ2, and (c) *E. coli* ATCC25922. Quantification (upper panel) and images (lower panel) of the biomass of a 48 h biofilm adhering in micro-titer plates; optical density after staining with 0.1% safranin was measured at 540 nm.



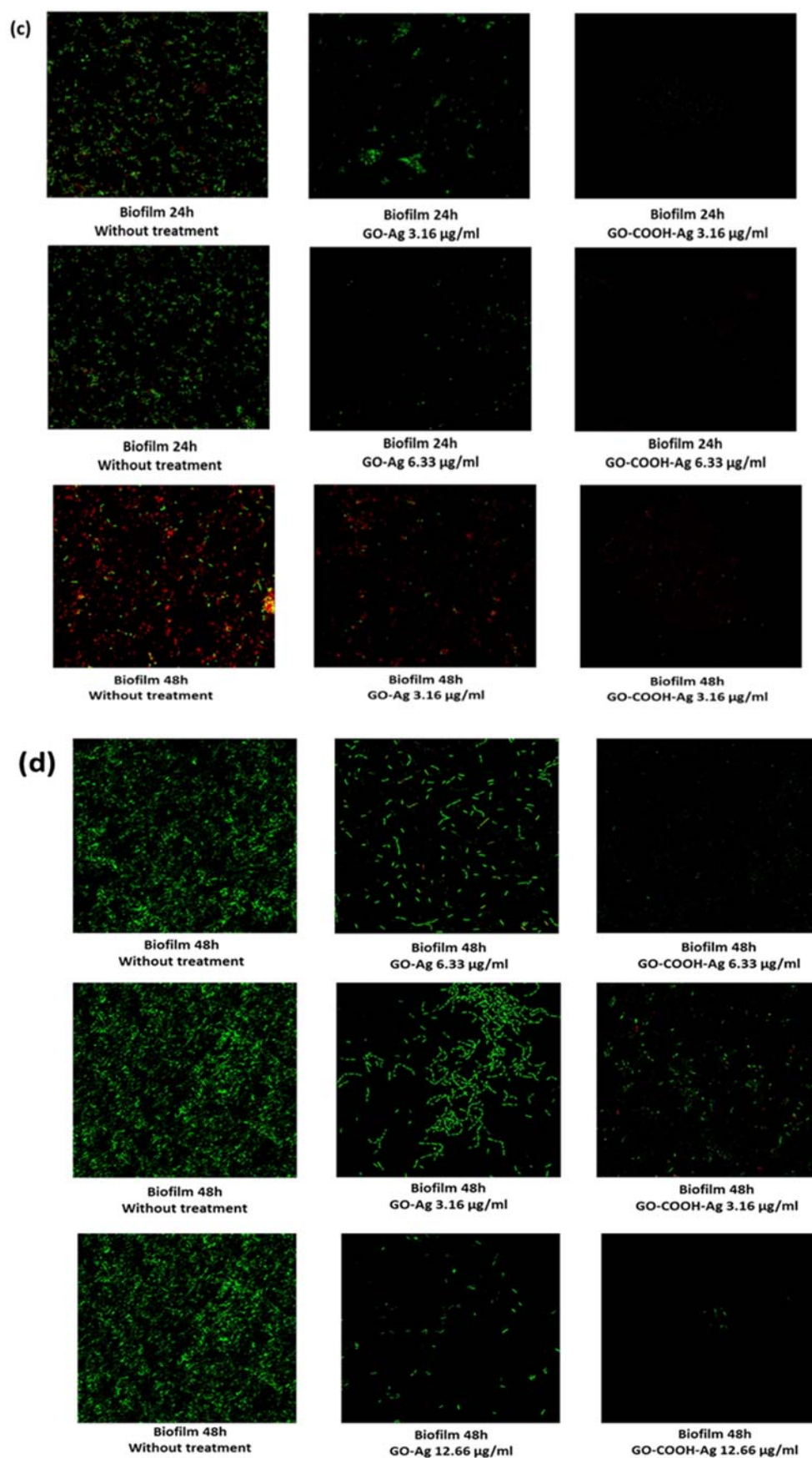


Figure S11. Confocal microscopy images biofilm (40× oil objective) of (a) *S. aureus* V329, (b) *S. epidermidis* RP62A, (c) *P. aeruginosa* PFQ2, and (d) *E. coli* ATCC25922.