

Supplementary data

Inhibition of Wild *Enterobacter cloacae* Biofilm Formation by Nanostructured Graphene- and Hexagonal Boron Nitride-Coated Surfaces

Elsie Zurob ^{1,2}, Geraldine Dennett ¹, Dana Gentil ¹, Francisco Montero-Silva ¹, Ulrike Gerber ³, Pamela Naulín ⁴, Andrea Gómez ⁴, Raúl Fuentes ⁵, Sheila Lascano ⁶, Thiago Henrique Rodrigues da Cunha ⁷, Cristian Ramírez ⁸, Ricardo Henríquez ⁹, Valeria del Campo ⁹, Nelson Barrera ⁴, Marcela Wilkens ² and Carolina Parra ^{1,*}

¹ Laboratorio Nanobiomateriales, Departamento de Física, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso, Chile; elsie.zurob@usach.cl (E.Z.); g.dennett@gmail.com (G.D.); dana.gentil@usm.cl (D.G.); monteroster@gmail.com (F.M.-S.)

² Laboratorio de Microbiología Básica y Aplicada, Universidad de Santiago de Chile, Avenida Libertador Bernardo O'Higgins 3363, Santiago, Chile; marcela.wilkens@usach.cl

³ Faculty Environment and Natural Science, Institute of Biotechnology, Brandenburg University of Technology, Universitätsplatz 1, 01968 Senftenberg, Germany; gerberu@b-tu.de

⁴ Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile; pnaulin@uc.cl (P.N.); agomez@bio.puc.cl (A.G.); nbarrera@bio.puc.cl (N.B.)

⁵ Departamento de Industrias, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso, Chile; raul.fuentes@usm.cl

⁶ Departamento de Mecánica, Universidad Técnica Federico Santa María, Avda. Vicuña Mackenna 3939, Santiago, Chile; sheila.lascano@usm.cl

⁷ Departamento de Física, CTNanotubos, Universidade Federal de Minas Gerais, Belo Horizonte 31310260, Brazil; thiago.curha@ctnano.com.br

⁸ Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso, Chile; cristian.ramirez@usm.cl

⁹ Departamento de Física, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso, Chile; ricardo.henriquez@usm.cl (R.H.); valeria.delcampo@usm.cl (V.d.C.)

* Correspondence: carolina.parra@usm.cl

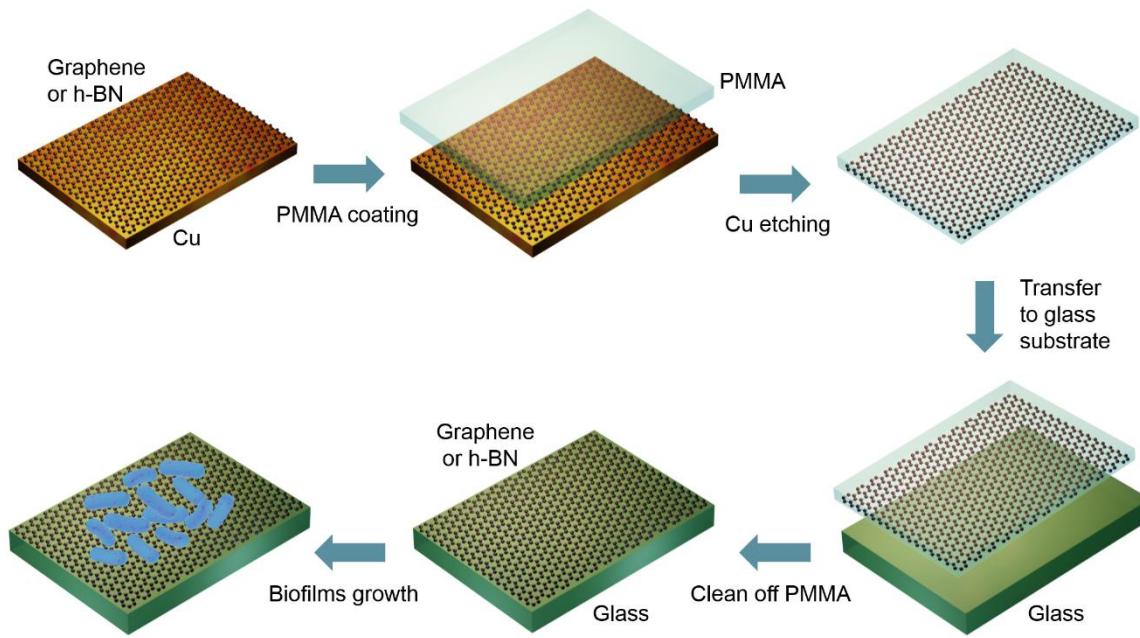


Figure S1. Illustrative diagram showing the PMMA-assisted transfer method used to obtain graphene- and h-BN-coated glass samples for the present study. A thin layer of PMMA (Polymethyl methacrylate) on graphene, on Cu (and h-BN on Cu) foil was produced by spin coating. The polymer provides a supportive framework for the two-dimensional (2D) coating before the transfer. The Cu substrate underneath was then etched away using an ammonium persulfate ($(\text{NH}_4)_2\text{SO}_8$) solution. After the Cu foil was completely dissolved, the floating membrane was scooped and placed onto glass. After drying, the polymeric film was dissolved with acetone.

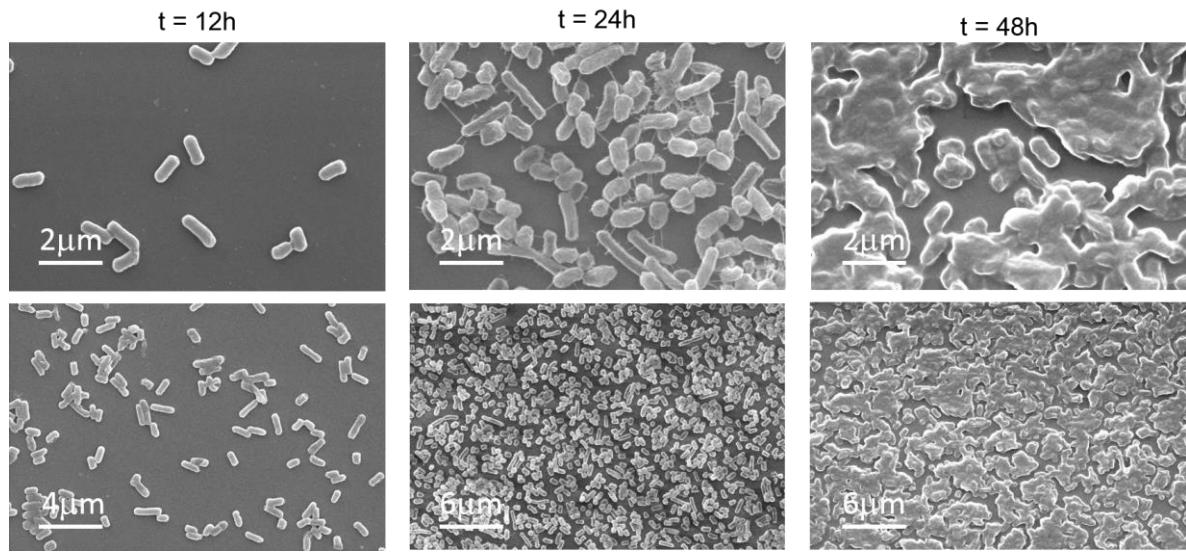


Figure S2. SEM image of *E. cloacae* after 12 h, 24h and 48h incubation on a glass surface. Reversible stage of biofilm formation can be observed by, first, the presence of bacteria adhered to the surface (12h) and then the production of filaments that assist a robust bacterial adhesion (24h). After 48 h incubation on a glass surface, the irreversible stage of biofilm growth is reached with bacteria encapsulated in exopolymeric substances (EPS).

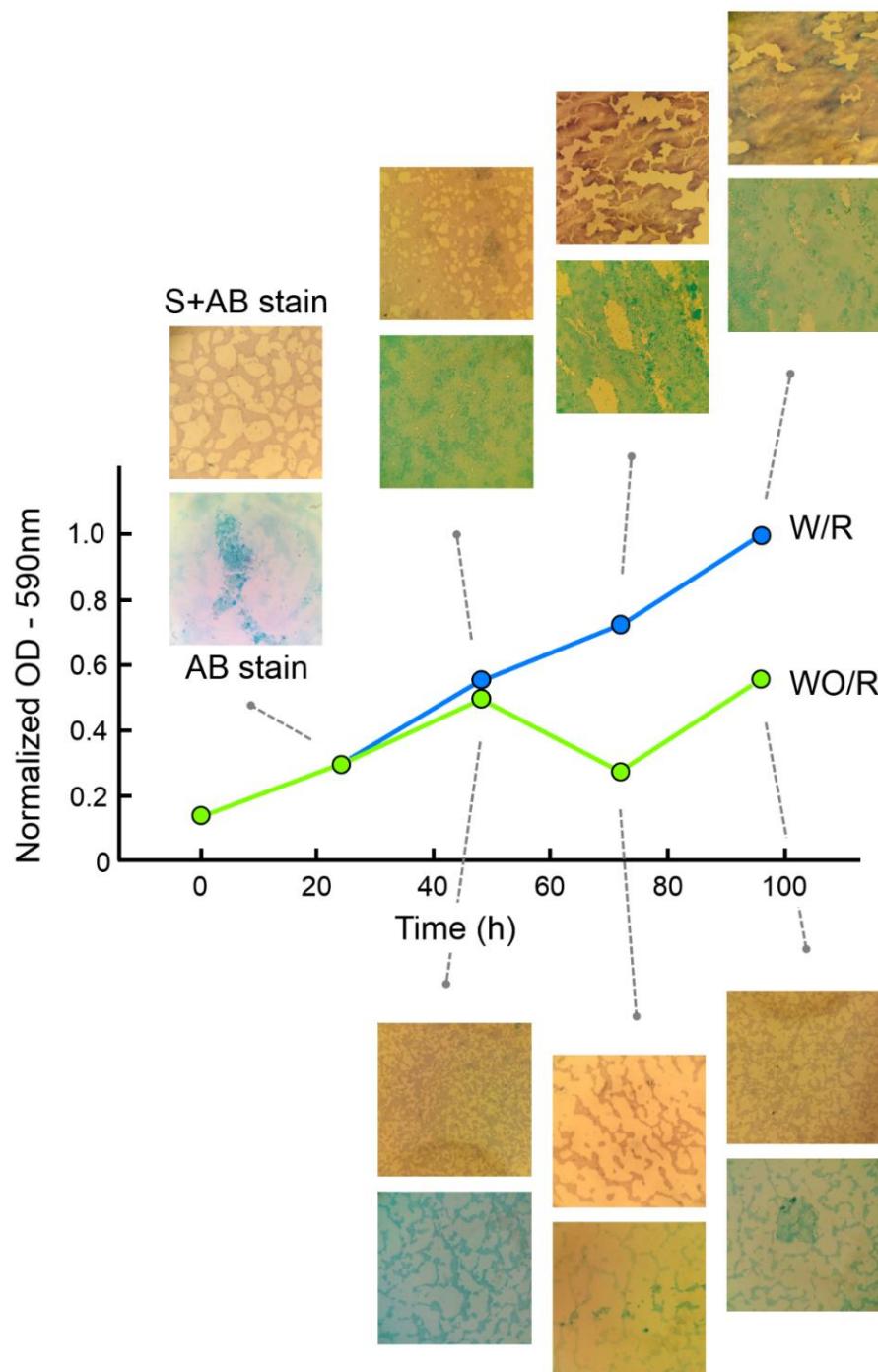


Figure S3. Optical density (OD) measurements and staining test of *E. cloacae* biofilms grown on glass as a function of time. The response of biofilms under no media replacement incubation conditions (WO/R) and media replacement incubation conditions (W/R) is depicted. Corresponding staining test for each time are included (WO/R and W/R).

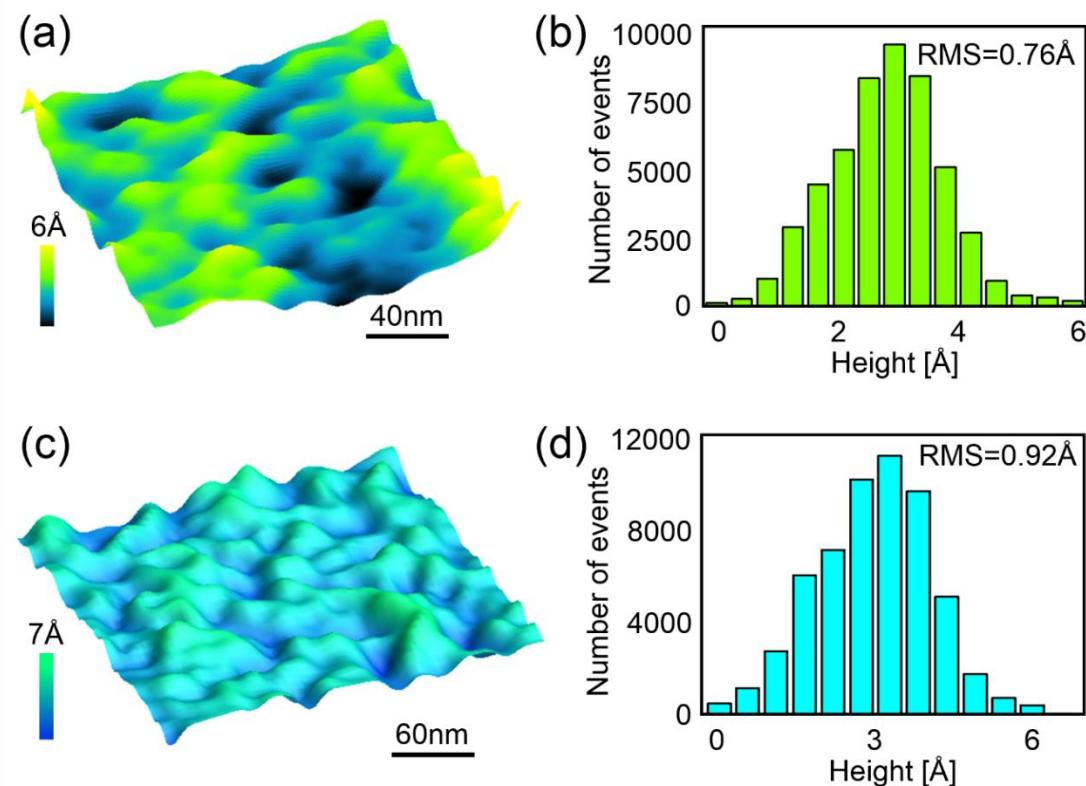


Figure S4. AFM image of graphene-coated glass sample (a) and h-BN-coated glass sample, (c) with their respective roughness analyses; (b) and (d).

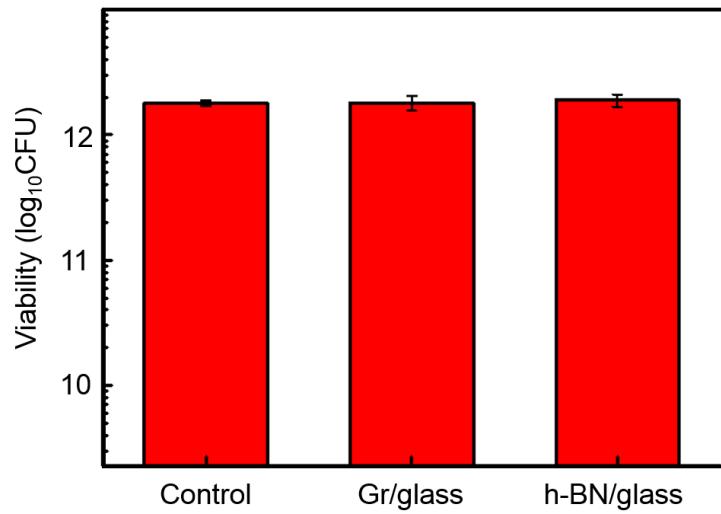


Figure S5. Cell viability of *E. cloacae* in planktonic state (not adhered) recovered after 48h exposure to graphene- and h-BN-coated glass.

Table S1. Morphological and biochemical test performed for strain identification of wild *Enterobacter cloacae*

Test or characteristic	Result	
	<i>E. cloacae</i> ATCC 13047	Wild isolate
Gram stain reaction	-	-
Cell morphology	Rod	Rod
Motility	+	+
Growth on MacConkey agar	+	+
Colonies	Pink	Pink
Precipitation of bile salts	-	-
Lactose fermentation	+	+
Glucose	+	+
Gas from glucose	+	+
Production of H ₂ S	-	-
Lysine decarboxylase	-	-
Ornithine decarboxylase	+	+
Simmons citrate	+	+
Indol	-	-
Oxidase	-	-
Catalase	+	+

+ = positive, - = negative