EFFECT OF AgNPs AND AgNPs-GO ON THE BIOADHESION OF *Pseudomonas aeruginosa* ON STAINLESS STEEL SURFACES.

Silvia E. Rastelli[1,2], Carolina Angulo-Pineda[3], Humberto Palza[3], Marisa R. Viera[1,4], Sandra G. Gómez De Saravia[1,2]

(1) Centro de Investigación y Desarrollo en Tecnología de Pinturas (CIDEPINT), CICPBA-CONICET-CCT, La Plata-UNLP. 52 e/ 121 y 122 (1900), La Plata, Buenos Aires, Argentina.
(2) Facultad de Ciencias Naturales y Museo, UNLP. Av.60 esq. 122 (1900), La Plata, Buenos Aires, Argentina.
(3) Laboratorio de Ingeniería de Polímeros, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, A. Blanco Encalada 2085, Santiago, Chile.
(4) Facultad de Ciencias Exactas, UNLP. Calle 47 esq.115, (1900), La Plata, Buenos Aires, Argentina. e.rastelli@cidepint.gov.ar

With the aim of incorporating nanohybrid materials in antimicrobial coatings, the inhibition of the adhesion of *Pseudomonas aeruginosa* on 430 AISI stainless steel coupons exposed to AgNPs and AgNPs-GO solutions was studied. AgNPs were obtained by green synthesis, while AgNPs-GO by *ex-situ* and *in-situ* synthesis. Coupons were immersed in these solutions, to form a coating. Bacterial adherence values obtained were: 10^5-10^6 CFU.cm^{-2} for coupons without coating; 10^4-10^5 CFU.cm^{-2} for AgNPs-GO (in-situ) coupons and values lower than 10^2 CFU.cm^{-2} on AgNPs and AgNPs-GO (ex-situ) coupons. These results were corroborated by scanning electron microscopy observations.

The formation of biofilms by *Pseudomonas aeruginosa* (*P. aeruginosa*) is related to the presence of both flagellar and pili-mediated motilities [1], the production of large amounts of extracellular polymeric substances (EPS) [2] and the quorum sensing system that controls the cell-cell signaling processes. The removal and eradication of biofilms is generally achieved by mechanical force, acid- or alkaline-based detergents or chemical disinfectants. However, the efficiency of these chemical products is strongly affected by factors such as pH, temperature, solubility, concentration and exposure time [3]. One of the most effective strategies for the prevention of microbial colonization is to develop a functional material with highly antimicrobial properties. Recently, the antimicrobial efficacy of engineered nanoparticles (NPs) including metal and carbon-based NPs has been widely studied [4,5]. Among the great variety of antibacterial materials, silver NPs (AgNPs) are marked out as antimicrobial reagents with high capability due to their large surface area and slow release properties [6,7]. Silver nanoparticles assembled on graphene oxide (GO) sheets have been exploited as novel antibacterial systems [8]. Although the potential for these nanocomposites to prevent biofilm formation has not been explored and the antimicrobial effect AgNPs and GO is known, the development of hybrids materials of AgNPs-GO has considerable interest in various applications since they exhibit synergistic bactericidal properties that exceed the yields of the individual components.

For the green synthesis of the AgNPs from a silver nitrate solution ($AgNO_3$), gelatin (Sigma Aldrich) was used as reducing and stabilizing agent. A certain amount of gelatin was prepared with DI water at 60 °C for 30 min [9]. The gelatin dispersion was added dropwise to the $AgNO_3$ solution (0.1 M) and then the final solution was stirred gently for a couple of minutes (Fig. 1 a). For the *ex-situ* synthesis of the hybrid material, 0.1 g of GO was dispersed in 30 mL of DI water, sonicated during 30 min. Finally, the GO and the AgNPs were mixed and sonicated for 60 min (Fig. 1 b).

For the *in-situ* synthesis, a homogenous solution of GO was mixed with a 0.1M $AgNO_3$ solution. After that, the temperature was raised to 100ºC. At this time, sodium citrate (0.1

mM) was added dropwise until the solution turned gray [10]. AgNPs anchored in the surface of the GO were obtained as shown in the spectra from Fig. 1c [9, 10].

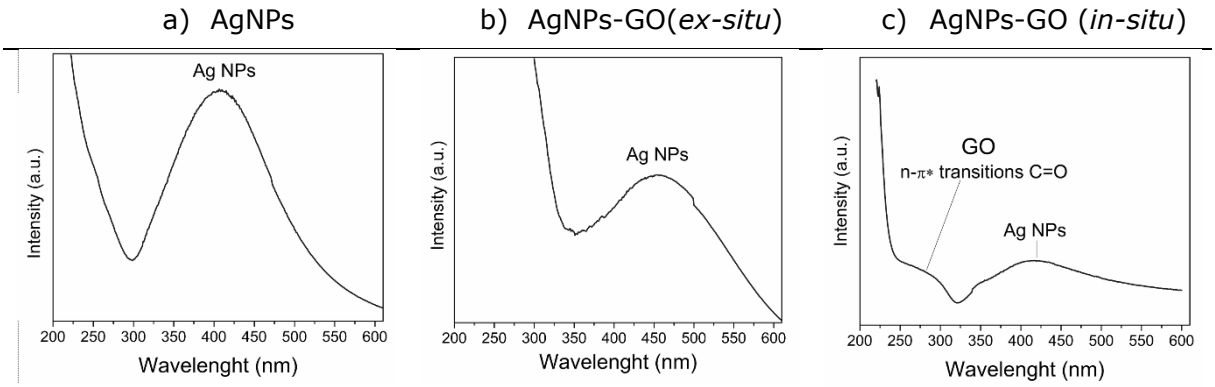
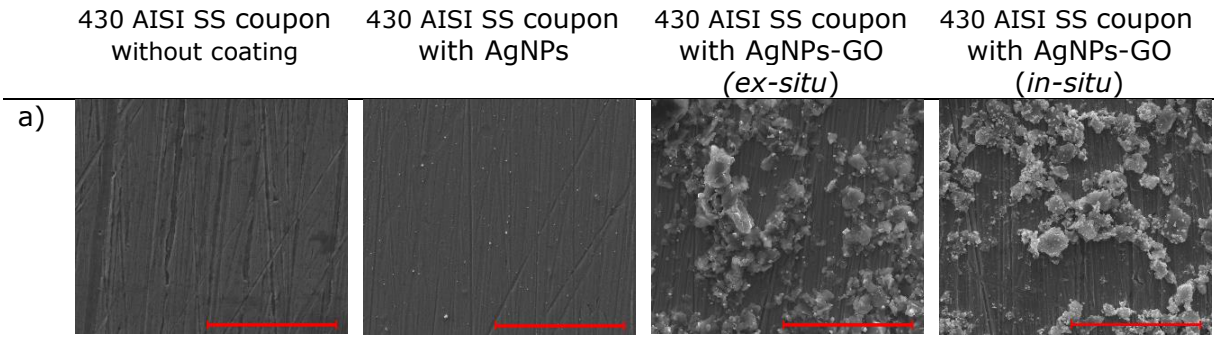


Figure 1. UV-visible spectra of a) AgNPs by green synthesis, b) AgNPsGO *ex-situ* and c) *in-situ* synthesis.

AISI 430 Stainless steel (SS) coupons, previously sterilized with UV light, were immersed in AgNPs and nanohybrids solutions for 24 h at 4 °C, to form a coating. Then, coupons were removed from the solutions and dried in the laminar flow bench. The contact angle on the SS coupons with the different coatings was measured by the drop method using an optical microscope with an image analyzer. Inhibition of bacterial adhesion was evaluated in multi-well plates. In each well, 1 mL of the *P. aeruginosa* inoculum with an OD (600nm) ≈ 0.1 (≈ 10^8 CFU.mL^{-1}) and coupons with and without coatings were placed. All the experiments were performed in duplicated. The coupons remained in the culture for 24 h at 28-30 °C. After that time, one coupon of each condition was used to perform bacterial plate counts and another one was used for scanning electron microscopy (SEM) observations previous fixation, dehydration and metallization.

No significant differences were observed in the contact angle measured among SS samples with and without coatings. The angles values were smaller than 90 degrees, pointing out the hydrophilic character of the surfaces.

The number of *P. aeruginosa* cells adhered on the coupons were 10^5-10^6 CFU.cm^{-2} for control coupons, 10^4-10^5 CFU.cm^{-2} for AgNPs-GO (*in-situ*) coupons and values lower than 10^2 CFU.cm-^{2} on AgNPs and AgNPs-GO (*ex-situ*) coupons, indicating that AgNPs and AgNPs-GO (*ex-situ*) possesses antifouling activity. These results were corroborated by the SEM observations (Fig. 2).



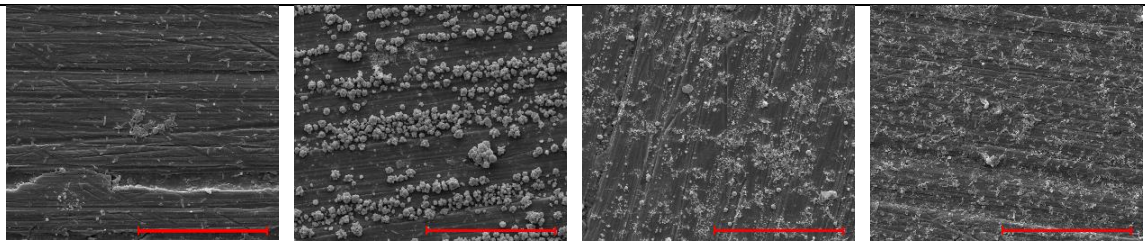


Figure 2. SEM micrographs (mag.3500 x) showing the aspect of 430 AISI stainless steel coupons *a)* before and *b)* after exposure to *P. aeruginosa* culture and EDX analysis. Scale bar: 40 μm. EDX values are in percentage.

Similar results regarding the enhanced antibacterial activities of AgNPs-GO vs. AgNPs were also reported by Zhang *et al.* [11] who studied their effect against both Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis*. Kalishwaralal *et al.* [12] described the *in vitro* activity of biologically synthesized AgNps on biofilms formed by *P. aeruginosa*, and their results showed more than 95 % inhibition of biofilm formation.

The GO sheets play an important role as a supporting and stabilizing agent, preventing the agglomeration of the silver nanoparticles and consequently a reduction of the antibacterial activity [10].

Our preliminary results support the idea that GO-Ag nanocomposites may be applied in antimicrobial coatings to prevent the development of biofilms.

Acknowledgements: The authors are grateful to the National University of La Plata (Project 11/ I201); CONICET (PIP No. 00314); CICBA (602/16) for the grants received to finance this work; and to Eng. P. Seré (ANELPIRE, CIDEPINT) for the contact angle measurements.

References

[1] S.S. Branda, A. Vilk, L. Friedman, R. Kolter, Biofilms: The matrix revisited, Trends Microbiology 13, 2005, 20–26.

[2] R.A.N. Chmielewski, J.F. Frank, Biofilm formation and control in food processing facilities, Comprehensive Reviews in Food Science and Food Safety 2, 2003, 22–32.

[3] R.V. Houdt, C.W. Michiels, Biofilm formation and the food industry, a focus on the bacterial outer surface, Journal of Applied Microbiology 109, 2010, 1117–1131.

[4] X. Liang, M.Sun, M.L.Li, R.Qiao, K.Chen, Q.Xiao, F.Xu, Preparation and antibacterial activities ofpolyaniline/$Cu_{0.05}Zn_{0.95}O$ nanocomposites, Dalton Transaction 41, 2012, 2804-2811.

[5] S. Liu, M.Hu, T.H.Zeng, R.Wu, R.Jiang, J.Wei, L.Wang, J.Kong, Y.Chen, Lateral dimension-dependent antibacterial activity of graphene oxide sheets, Langmuir 28, 2012, 12364–12372.

[6] A. Taglietti, Y.A.Diaz Fernández, E.Amato, L.Cucca, G.Dacarro, P.Grisoli, V. Necchi, P.Pallavicini, L.Pasotti, M.Patrini, Antibacterial activity of glutathione-coated silver nanoparticles against Gram positive and Gram negative bacteria, Langmuir28, 2012, 8140–8148.

[7] M. Rai, A. Yadav, A.Gade, Silver nanoparticles as a new generation of antimicrobials, Biotechnology Advances, 27, 2009, 76–83.

[8] L. Liu, Y. Wang, X. Yan, D.D. Sun, Facile synthesis of monodispersed silvernanoparticles on graphene oxide sheets with enhanced antibacterial activity, New Journal of Chemistry 35, 2011 1418–1423.

[9] D. Zhang, X. Liu, X. Wang, Green synthesis of graphene oxide sheets decorated by silver nanoprisms and their anti-bacterial properties, Journal of Inorganic Biochemistry105, 2011, 1181-1186.

[10] A. de Faria, D. Martinez, S. Meira, A. de Moraes, A. Brandelli, A. Souza Filho y O. Alves, Anti-adhesion and antibacterial activity of silver nanoparticles supported on graphene oxide sheets, Colloids and Surfaces B: Biointerfaces, 113, 2014, 115-124.

[11] H. Zhang, G. Grüner, Y. Zhao,Recent advancements of graphene in biomedicineJournal of Material Chemistry B 20, 2013, 2542–2567.

[12] K.Kalishwaralal, S. B. ManiKanth, S. R. K.Pandian,V. Deepak, S.Gurunathan, Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis, Colloids and Surfaces B: Biointerfaces, 79, 2010, 340–34.