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Conservação | Conservation

Silver/dioxide titanium nanocomposites as biocidal treatments on limestones

Javier Becerra, Ana Paula Zaderenko Partida, Pilar Ortiz Calderón

Abstract: Biodegradation of stones is a common and undesirable alteration in historical buildings. Restorers have been using different treatments, especially chemical methods, to prevent biodeterioration. These treatments often have disadvantages such as low long-term effectiveness, high toxicity on human health and the environment and/or physicochemical incompatibility with the original stone (chromatic alteration or chemical degradation).

In this research, different biocidal treatments based on silver and titanium dioxide nanocomposites have been tested on limestones from Utrera's quarry (Seville, Spain), a stone employed in historical buildings in the south of Spain. Two AgNPs syntheses have been studied; the principal difference between them was the use of trisodium citrate as stabilizer.

Optimum nanocomposite composition and dosage to minimize chromatic alteration after application of treatments without cut down the biocide effectiveness have been set up. Treatments based on silver-titanium dioxide (Ag/TiO_2) nanocomposites stabilized with citrate have been able to keep clean the limestone due to the biopatina formation reduction and the surface color change has been below 10%.

Key words: Silver/titanium dioxide nanocomposite, Biocide, Conservation, Limestone.

Nanocompuestos de plata / dióxido de titanio como tratamientos biocidas sobre calizas

Resumen: La biodegradación en piedra es una alteración común e indeseable en los edificios históricos. Los restauradores han estado utilizando diferentes tratamientos para la prevención del biodeterioro, especialmente métodos químicos. Estos tratamientos suelen tener ciertas desventajas, tales como baja durabilidad a largo plazo, alta toxicidad para el hombre y el medioambiente y/o su incompatibilidad físico-química con la piedra original (alteración cromática o degradación química).

En esta investigación, diferentes tratamientos biocidas basados en nanopartículas de plata y dióxido de titanio han sido probados sobre calizas procedentes de la cantera de Utrera (Sevilla, España), piedra utilizada en la construcción de diferentes edificios históricos del sur de España. Para ello, dos síntesis de nanopartículas de plata han sido utilizadas, cuya principal diferencia estriba en el empleo de citrato sódico como estabilizante.

La óptima composición y dosis de nanocompuestos que logra disminuir los incrementos de color del tratamiento sin mermar sus propiedades como biocida ha sido investigada. El nanocompuesto de plata/dióxido de titanio estabilizado con citrato ha permitido mantener limpia la piedra caliza, reduciendo la formación de biopátina y generando un cambio de color tras el ensayo menor del 10%.

Palabras clave: Nanocompuesto de plata/dióxido de titanio, Biocida, Conservación, Caliza.

Introduction

Biopatina formation on stones is a common pathology of historic buildings. The most common microorganisms forming biopatinas are bacteria, algae and fungi. They can cause physical impact but also chemical processes due to organic acids produced during their metabolism. These organic acids can be responsible of solubilisation or chelation of different minerals present in stone composition, as a consequence pitting, sanding and peeling may appear in stones. Apart from that, biopatina on stone surfaces can change the diffusion of water vapour into the material and in the capillary water uptake, although chromatic changes caused by a series of biogenic pigments are the most evident alterations observable by the naked eye (Gaylarde et al. 2003). Current chemical treatments used to avoid biodeterioration (quaternary ammonium compounds, phenolic compounds, organimetallic compounds, or urea derivates) often have low durability, high toxicity and/or cause interaction on stone materials such as chromatic alterations, dissolution of calcite or oxidation of minerals induced by different additives or solvents (Nugari and Salvadori 2002).

The recent advances in nanotechnology are enabling us to develop new treatments to avoid microbial effects on the stone, such as the application of silver nanoparticles (AgNPs) which have biocidal properties (Lok et al. 2007; Sondi and Salopek-Sondi 2004) or titanium dioxide nanoparticles (TiO_2NPs) which have antibacterial properties (Foster et al. 2011) and high photocatalytic activity at ultraviolet light (Munafó et al. 2015). The mixture of the cited two types of nanoparticles allows us to create new nanocomposites combining both properties. Moreover, the reactivity of TiO_2NPs at visible spectrum is increasing by the Ag nanoparticles such as it has been demonstrated by Zhao et al. 2012. The biocide effects of silver/titanium dioxide nanocomposites have been checked for different applications such as medical devices, water filters, textile or leather (Yaşa et al. 2012; Lungu et al. 2014). The aim of this paper is to synthesize, characterize and evaluate different nanocomposites containing silver and titanium dioxide as potential biocidal treatment for stone conservation in Cultural Heritage.

Methods

—*Synthesis and characterization of nanocomposites*

Two different silver-based syntheses based on a bottom-up method have been employed for this study. The silver nanoparticles obtained were applied alone or with other nanoparticles such as titanium dioxide and activated carbon.

The first synthesis of silver is according to Flores et al. (2010) and contains trisodium citrate as stabilizer of silver nanoparticles. The second synthesis of silver is according to Caro et al. (2015) and does not contain any stabilizer for silver and the reducing agent used was sodium borohydride.

Ag@cit and Ag/TiO_2@cit were made with silver nanoparticles stabilized with trisodium citrate (Flores et al., 2010). Ag/TiO_2/AC, Ag/TiO_2, and ¼Ag/TiO_2 were made with silver nanoparticles stabilized without trisodium citrate (Caro et al. 2015).

The table 1 shows composition of the six different types of nanocomposites with Ag, TiO_2, citrate or active carbon employed. The silver concentration varies between 0.05-0.02 mg/mL, TiO_2 between 0.06-0.12 mg/mL and active carbon was included only in one of the products at 0.83 mg/mL concentration. TiO_2 nanoparticles were also tested in order to compare with the mixtures.

A Bruker Senterra confocal Raman spectroscope was employed to analyze the composition of the six synthesized nanocomposites.

The physiochemical characterization of nanocomposites has been realized using UV-Vis spectroscopy, hydrodynamic diameter and zeta potential measurements. UV-Vis spectroscopy carried out with an Ocean optics spectrometer equipped with a HR4000 detector allows us to study silver plasmon. Hydrodynamic diameter and zeta potential measurements were carried out using a Dynamic Light Scattering Zetatrac Analyzer. These properties allow us to check the stability of the product: hydrodynamic diameter is an estimation of diameters due to relationship

Table 1.- Nanocomposites composition and concentrations.

	Nanocomposites and Nanoparticles					
	TiO_2	Ag/TiO_2/AC	Ag/TiO_2	¼Ag/TiO_2	Ag@cit	Ag/TiO_2@cit
Ag (mg/mL)	-	0.02	0.02	0.005	0.005	0.005
TiO_2 (mg/mL)	✓	0.12	0.12	0.12	-	0.06
AC (mg/mL)	-	0.83	-	-	-	-
Citrate	-	-	-	-	✓	✓
Concentration for assays	0.53	0.22*	0.20*	0.66*	0.03*	0.16*

*(equalized at 0.03 Ag mg/mL)

between nanoparticles and solvents, and zeta potential (ZP) is a measurement of dispersion stability. Dispersions of nanoparticles with zeta potential (ZP) over 30 mV are more stable according to Koutsoukos et al. (2006), those nanocomposites with zeta potential between 20 and 30 mV may aggregate, and zeta potential under 20 mV may be considered more unstable dispersions.

—*Antibacterial activity*

Antibacterial activity was tested in liquid cultures by growth curves. This assay consisted in determining the growth of the bacteria E*scherichia coli* (*E. coli*) without (control) and with different amounts of nanocomposites. Absorbance was measured at 600 nm using a flourimeter POLARstar. 200 µl of *E. coli* inoculums at an optical density of 0.3 and different aliquots (4, 10, 20 and 40 µL) of the tested nanocomposites were employed to these assays. Table 1 shows the nanocomposites and their concentrations. The final nanocomposite concentration (Table 1) was calculated in base of the amount of silver nanoparticles present in each nanocomposite. In this way, the amount of silver nanoparticles has been equalized at 0.03 mg/mL (except TiO_2), so the concentrations of total nanocomposite vary between 0.03 mg/mL (Ag@cit nanoparticle) and 0.66 mg/mL (¼Ag/TiO_2 where the amount of Ag used in the synthesis process was lower and so the presence of TiO_2 is higher). The assays were carried out under continuous shaking at 37 ºC.

—*Biocide assay on limestone of Utrera*

Limestone slabs from Utrera's quarry (Seville, Spain) were selected for the assay of inhibition of biopatina formation because the use of this quarry is well-known employed in Sevillian historical buildings. Following the characterization made by Guerrero Montes (1990) for this limestone, it has a high content of quartz and fossils (2-5%) with a porous size between 0.1-1 µm. This limestone has medium-high porosity (9.6%) according to Ortiz et al. (2008). The size of the slabs was 1.5 x 1.5 x 0.5 cm. 200 µL of nanocomposites at the concentrations specified in the table 1 were deposited over the limestone slab surface. The concentrations employed were calculated equalizing the amount of AgNPs for each nanocomposite, except TiO_2.

Clorophytes coming from biopatinas of different Sevillian monuments were cultivated in a phosphate medium. Biopatina formations on stone were generated by immersion of the limestone slabs. The ambient conditions during the assay were room temperature and lighting with an incandescent lamp. Biopatina formations on stones were measured by a Colorimeter X-Rite SP20after 28 days of immersion and 5 days at room temperature for drying. The CIELab colour-system has been used in this assay, L* describe the brightness and a* and b* refer to the red-green and yellow-blue colour tonalities, respectively.

The Biopatina Formation Inhibition (BFI) was quantified in terms of total colour difference ($\Delta E^*_{BFI} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{½}$), defined as the distance between the initial and final points in the CIELab Colour sphere during the processes of biobatina formation, where $\Delta L^* = L_{after\ assay} - L_{before\ assay}$, $\Delta a^* = a_{after\ assay} - a_{before\ assay}$ and $\Delta b^* = b_{after\ assay} - b_{before\ assay}$. *The results have been classified as optimal if colour cha*nge (BFI) is less that 5 (it could not been distinguished by naked eyes), intermediate in case of total colour change between 5-20, and negative if total color change is over 20.

Conservation treatments should not change the appearance of stones in cultural heritage. The total colour change (TCC) due to the application of nanocomposites and formation of biopatina has also been measured. Total colour change ($\Delta E^*_{TCC} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{½}$), is defined as the distance between the initial and final points in the CIELab Colour sphere with and without treatment and biopatina formation, where $\Delta L^* = L_{with\ treatment\ and\ biopatina} - L_{without\ treatment\ and\ biopatina}$, $\Delta a^* = a^*_{with\ treatment\ and\ biopatina} - a^*_{without\ treatment\ and\ biopatina}$ and $\Delta b^* = b^*_{with\ treatment\ and\ biopatina} - b^*_{without\ treatment\ and\ biopatina}$. The optimal value of $\Delta E^*_{TCC} < 10$ described by Ortiz et al. (2013) for cleaning of stones were used as reference in this assay. These results have been classified as optimal if total colour change (TCC) is less that 10, intermediate in case of total colour change between 10-20, and negative if total color change is over 20.

Results and discussion

—*Nanocomposites characterization*

Raman spectra show the chemical composition of the different nanocomposites. The Raman spectra of the different pure compounds used in the synthesis have been studied to make the comparison with the Raman spectrum of the different nanocomposites synthesized. Figure 1(blue spectrum) shows the Raman scattering spectrum of the pure TiO_2, which bands are situated at 200, 400, 500 and 600 cm^{-1}. These peaks can be assigned to the typical molecular vibration mode of the TiO_2 in anatase phase (Xu et al. 2012). The TiO_2 band at 200 cm^{-1} meets with a typical band of the Ag, but the principal difference is that Ag band is more prominent. In the case of pure activated carbon, Figure 1 (brown spectrum) shows two prominent band at 1300 and 1600 cm^{-1}. The first nanocomposite studied was Ag/TiO_2/AC [Figure 1. purple spectrum]. The Raman spectrum of this nanocomposite shows the prominent silver band at 200 cm^{-1} while the TiO_2 (200, 400, 500 and 600 cm^{-1}) and AC (1300 and 1600 cm^{-1}) bands are more lightly, In the case of the Raman spectrum of Ag/TiO_2 nanocomposite [Figure 1. sky-blue spectrum], it can be observed the bands of the Ag and TiO_2 nanoparticles.

The principal different between the two synthesises employed in this research corresponds to the use of trisodium citrate as stabilizer of the silver nanoparticle. Figure 1 (green spectrum) shows the Raman spectrum

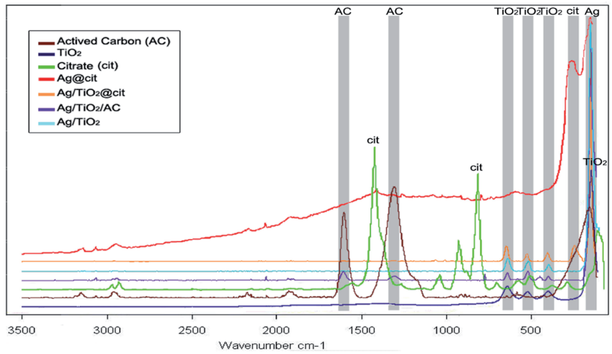

Figure 1.- Raman spectra: TiO_2, AC, Sodium citrate, Ag/TiO_2/CA, Ag/TiO_2, Ag@cit and Ag/TiO_2@cit.

of pure trisodium citrate with highlight bands at 250, 800 and 1500 cm^{-1}. However, the Raman spectrum of the Ag nanoparticle stabilized with citrate (Ag@cit) shows only a prominent citrate band at 250 cm^{-1} and the Ag band at 200 cm^{-1} [Figure 1.red spectrum]. Ag/TiO_2@cit nanocomposite [Figure 1. orange spectrum] shows the bands corresponding with Ag, TiO_2 and citrate.

Silver plasmon is a special property of AgNPs. The shape and amplitude of the silver plasmon determined by UV-Vis spectroscopy is a way to assess dispersion of nanocomposite size. Higher bandwidth implies particles with different sizes and less stability of dispersion.

Nanocomposites stabilized with citrate show a narrow bandwidth at 400 nm, result of their less particle size distribution (polydispersity). Nanocomposites without citrate show a displacement of the band. Their bandwidths are larger due to their less stability and the increase of polydispersity.

Table 2 shows a summary of the stability parameters of the nanocomposites synthesized in this research. Hydrodynamic diameter (HD) is closely related to the size of nanoparticles plus its interaction with solvent molecules. Nanocomposites stabilized with citrate show the lowest diameter and polydispersity, with sizes less than 45 nm. However, nanocomposites without citrate showed a higher polydispersity, and diameters with values higher than 100 nm.

Hydrodynamic diameters of the different nanocomposites have been classified according to the pore size of Utrera´s limestones [Table 2]. In consonance with this classification, Ag@cit and Ag/TiO_2@cit nanocomposites have the best penetration through the porous system due to the hydrodynamic diameters of these two nanocomposites are lower than 0.1 µm and the pore size is since 0.1 µm. The rest of nanocomposites with HD over 0.1 µm could block up porous systems, generating aggregates and stains on surfaces.

Zeta potential (ZP) of 30mV is taken as a line between stable and unstable dispersions by Koutsoukos et al. (2006). According with this rule, nanocomposites stabilized with citrate are the most stable, with zeta potential values around 30mV. This means that these nanocomposites are lower capacity to form aggregates, especially in the case of Ag@cit nanoparticles [Table 2]. Nanocomposites without citrate show values lower than 20 mV and are more instable.

In summary, nanocomposites stabilized with citrate are more stable and have a less hydrodynamic size. Their stability permits to apply and conserve them in aqueous solvent. The solution is easily re-dispersed with hand-

Table 2.- Raman spectra: TiO_2, AC, Sodium citrate, Ag/TiO_2/CA, Ag/TiO_2, Ag@cit and Ag/TiO_2@cit.

	Nanoparticles and Nanocomposites					
	TiO_2	Ag/TiO_2/AC	Ag/TiO_2	¼Ag/TiO_2	Ag@cit	Ag/TiO_2@cit
Hydrodynamic Diameter (HD)[1]	+	+	+	+	+ +	+ +
Zeta Potential (ZP)[2]	- -	- -	- -	- -	+	-
Biopatina Formation Inhibition (BFI)[3]	+	-	-	+ +	+	+ +
Total Colour Change (TCC)[4]	-	-	-	-	+	+ +

[1] According to porous limestone sizes. (-): HD > 1 µm; (+): 0.1 < HD < 1 µm; (+ +): HD < 0.1 µm.
[2] According to Koutsoukos et al. (2006). (- -): ZP < 20 mV; (-): 20 < ZP < 30 mV; (+): ZP > 30 mV.
[3] According to total colour change of biopatina growth (ΔE^*_{BFI}). (-): BFI > 20; (+): 20 > BFI > 5; (+ +): BFI < 5.
[4] According to total colour change of nanocomposites and biopatina growth (ΔE^*_{TCC}). (-): TCC > 20; (+): 20 > TCC > 10; (+ +): TCC < 10.

shaking. Moreover, their fewer hydrodynamic diameters (HD) may facilitate the penetration through stone porous system.

— *Antibacterial activity*

All nanocomposites tested caused a decrease of E. coli growth in comparison with control samples without nanocomposites (sky-blue bars) [Figure 2]. The higher inhibition was generally associated to higher nanocomposite concentration, except Ag/TiO_2@cit and TiO_2. The best result was obtained with 40 µL of ¼Ag/TiO_2 nanocomposite where we observed a 40% decrease in bacterial growth. In contrast to this, Ag@cit nanoparticle showed lowest inhibition effects, especially at 10 µL and 20 µL. For this reason, we can confirm that the combinations of Ag/TiO_2 have best biocide properties than silver or titanium dioxide nanoparticles separately, the mixture of these two nanoparticles improve the biocide properties of silver and the catalytic properties of titanium dioxide (Zhao et al., 2012 and La Russa et al., 2014).

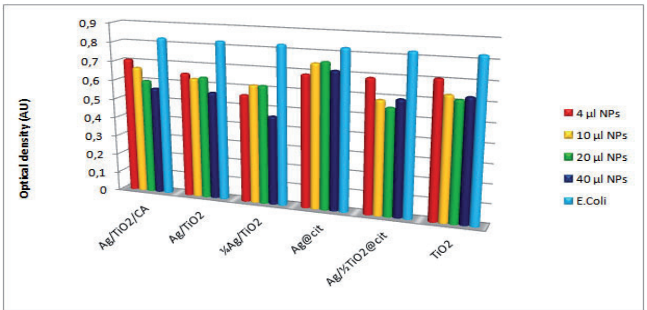

Figure 2.- E. Coli assays. Optical density at 7 h., when the stationary phase was initiated.

—Biocide assays on limestone of Utrera.

Biopatina Formation Inhibition (BFI) was estimated by comparison between surface colour before and after the trial. According to Prieto et al. (2004), this method is fast, easy and non destructive technique in comparison with Chlorophyll quantification or fluorescein diacetate hydrolysis. Moreover, this technique (ΔE^*) would allow us monitoring the biopatina inhibition on site.

All the nanocomposites generally allow us to reduce the biopatina formation, except in Ag/TiO_2/AC and Ag/TiO_2 [Table 02] where colour changes (ΔE^*_{BFI}) were over 20 (similar to control samples without nanocomposites). The colour change (ΔE^*_{BFI}) was below 5 in the slab with ¼Ag/TiO_2 and Ag/TiO_2@cit, so those nanocomposites showed the best biocide properties. It is important to note that the samples have been immersed in water during all the trial, generating optimal conditions for the growth of algae, but the stones in historical buildings are not immersed in water but subjected to frequent processes of hydration and drying. The Figure 3 shows slabs after assay and two slabs of control (d: with biopatina and e: untreated and unaltered sample). Biopatina was greenish [Figure 3.d], with the exception of the trials where the nanocomposites were applied and patina acquired orange colour due to the degradation of algal cells. Figures 3.a, b and c show the result of the assay in the slabs treated with the nanocomposites not stabilized with citrate. In this case, it can be observed as the algae colonies are degraded with the typical orange tone in the surface slabs while the Ag/TiO_2@cit nanocomposite [Figure 3.g] decrease the biopatina formation and the colour final of the slab is most similar to untreated and unaltered sample [Figure 03.e]. Total colour change (ΔE^*_{TCC}) shows the increment of colour caused by the treatment applied on the stone surface and the biopatina formation after the trial. This parameter tries to measure the applicability of these treatments for Cultural Heritage, so it takes in consideration the inhibition capacity of the different nanocomposites and the colour change generated by their applications. The best result was obtained for Ag/TiO2@cit nanocomposite. ($\Delta E^*_{TCC} < 10$) [Table 2]. In this case, although the comparison between the two better treatments (¼Ag/TiO_2 [Figure 3.c], Ag/TiO_2@cit [Figure 3.g]) allow us to corroborate the biopatina formation inhibition, the total colour change (ΔE^*_{TCC}) in the case of ¼Ag/TiO_2 is higher than 20 [Table 2] due to the darkening to the stone surface caused by the treatment. For this reason Ag/TiO_2@cit is the treatment recommended to use as biocide in Cultural Heritage, as it produce the best biocide effect with an admissible change of colour. Moreover, this Citrate-capped nanocomposite with TiO_2 (Ag/TiO_2@cit) showed the best efficiency because of lower concentration of this compound than nanocomposites without citrate was necessary for effective treatments [Table 1].

Figure 3.- Slabs after assay. (a) Ag/TiO_2/CA. (b) Ag/TiO_2. (c) ¼Ag/TiO_2. (d) Altered control slab. (e) Unaltered control slab. (f) Ag@cit. (g) Ag/TiO_2@cit. (h) TiO_2.

Conclusions

This preliminary evaluation of different Ag/TiO_2 nanocomposites showed good results as biocidal treatment. The mixtures of silver nanoparticles and titanium dioxide nanoparticles were more effective biocidal treatments than Ag or TiO_2 nanoparticles separately. The best results as restoration treatment were reported for nanocomposites stabilized with citrate because they have smaller particle size, are more stable colloids and exhibit excellent biocide properties inducing colour changes bellow 10%. Further studies are necessary in order to analyze different molar relation between Ag and TiO_2 in the protocol synthesis to establish the optimal treatment for stone on Cultural Heritage.

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Bibliography

CARO, C.; GÁMEZ, F.; SAYAGUES M. J.; POLVILLO, R. AND ROYO, J. L. (2015). "AgACTiO_2 Nanoparticles with Microbiocide Properties under Visible Light." *Materials Research Express*, n. 2, Vol. 5., 055002.

FLORES, C. Y.; DIAZ, C.; RUBERT, A.; BENÍTEZ, G. A.; MORENO, M. S.; FERNÁNDEZ LORENZO DE MELE, M. A.; SALVAREZZA, R. C.; SCHILARDI, P. L. AND VERICAT, C. (2010). "Spontaneous Adsorption of Silver Nanoparticles on Ti/TiO_2 Surfaces. Antibacterial Effect on Pseudomonas Aeruginosa." *Journal of Colloid and Interface Science*, n. 350, Vol. 2, pp. 402–408.

FOSTER, H.A.; DITTA, I.B.; VARGHESE, S. AND STEELE, A. (2011). "Photocatalytic desinfection using titanium dioxide: Spectrum and mechanism of antimicrobial activity" *Applied Microbiology and Biotechnology*, n 9, pp. 1847-1868.

GAYLARDE, C.; RIBAS SILVA, M. AND WARSCHEID, TH. (2003) "Microbial impact on building materials: an overview" *Materials and Structures*, n 36, , pp. 342-352.

GUERRERO MONTES, M. A. (1990). "Diagnóstico Del Estado de Alteración de La Piedra Del Palacio Consistorial de Sevilla. Causas y Mecanismos." University of Seville, 1990, pp. 15-19.

KOUTSOUKOS, P.K.; KLEPETSANIS, P. G. AND SPANOS, N. (2006) "Calculation of Zeta-Potentials from Electrokinetic Data." *Encyclopedia of Surface and Colloid Science*, New York, Taylor & Francis Group, pp. 1097–1113.

LA RUSSA, M.F.; MACCHIA, A.; RUFFOLO, S.A., DE LEO, F., BARBERIO, M., BARONE, P.; CRISCI, G.M. AND URZÌ, C. (2014)"Testing the antibacterial property of doped TiO_2 for preventing biodeterioration of Cultural Heritage building materials." *International Biodeterioration & Bioderadation*, n. 96, pp. 87-96.

LOK, C.N.; HO, C.M.; CHEN, R.; HE, Q.Y. et al. (2007) "Silver nanoparticles: partial oxidation and antibacterial activities" *Journal of Biological Inorganic Chemistry*., n. 12, pp. 527-534.

LUNGU, M.; GAVRILIU, Ş.; ENESCU, E.; ION, I.; BRĂTULESCU, A.; MIHĂESCU, G.; MĂRUŢESCU, L. AND CHIFIRIUC, M.C. "Silver-titanium dioxide nanocomposites as effective antimicrobial and antibiofilm agents" Journal of *Nanoparticle Research*, n. 16, 2014, 2203.

MUNAFÒ, P.; GOFFREDO, G. B.; AND QUAGLIARINI, E. (2015) "TiO2-Based Nanocoatings for Preserving Architectural Stone Surfaces: An Overview." *Construction and Building Materials*, n. 84, pp. 201–218.

NUGARI, M.P. AND SALVADORI, O. (2002) "Biocides and treatment of stone: Limitations and future prospects" *Art, Biology and Conservation. Biodeterioration of works of art*. The Metropolitan Museum, pp. 518-535.

ORTIZ, P.; ANTÚNEZ, V.; ORTIZ, R.; MARTÍN, J. M.; GÓMEZ, M. A.; HORTAL, A. R. AND MARTÍNEZ-HAYA, B. "Comparative Study of Pulsed Laser Cleaning Applied to Weathered Marble Surfaces." *Applied Surface Science*, n. 283, 2013, pp. 193–201.

ORTIZ, P.; GUERRERO, M. A.; VÁZQUEZ, M. A.; ORTIZ, R.; MARTÍN, J. M. AND PEÑA, M. C. (2008). "Accelerated Weathering Test as Environmental Behaviour Trials on Calcareous Stone." *Proceeding 11th International Congress on Deterioration and Conservation of Stone*., pp. 223–31.

SONDI, I. AND SALOPEK-SONDI, B. (2004). "Silver Nanoparticles as Antimicrobial Agent: A Case Study on E. Coli as a Model for Gram-Negative Bacteria." *Journal of Colloid and Interface Science*, n. 275, Vol. 1, pp. 177–82.

XU, J.; XIAO, X.; REN, F.; WU, W.; DAI, Z.; CAI, G.; ZHANG, S.; ZHOU, J.; MEI, F. AND JIANG, C. (2012). "Enhaced photocatalysis by coupling of anatase TiO_2 film to triangular Ag nanoparticle island" Nanoscale Research letters, n. 7, , pp. 239.

YAŞA, I.; LKHAGVAJAV, N.; KOIZHAIGANOVA, M.; ÇELIK, E. AND SARI Ö. (2012)"Assessment of antimicrobial activity of nanosized Ag doped TiO_2 colloids" *Word Journal of Microbiology and Biotechnology*, n. 28, pp. 2531-2539.

ZHAO, Y.; YANG, B.; XU, J.; FU, Z.; WU, M. AND LI, L. (2012)"Facile Synthesis of Ag Nanoparticles Supported on TiO_2 Inverse Opal with Enhanced Visible-Light Photocatalytic Activity." *Thin Solid Films*, n. 520, Vol. 9, pp. 3515–3522.

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Javier Becerra Luna
jbeclun@upo.es

Departamento de Sistemas Físicos, Químicos y Naturales. Universidad Pablo de Olavide, Ctra. Utrera, km 1, ES-41013, Seville, Spain.
Undergraduate Degree in Conservation and Restoration from Seville University with special mention. Now, Javier is researching about nanotechnology applied to Cultural Heritage for his PhD thesis at the Pablo de Olavide University in Seville, Spain. He has received several awards, highlighting Graduate Student Awards in the E-MRS Spring Meeting (2016).

Ana P. Zaderenko Partida
anzadpar@upo.es

Departamento de Sistemas Físicos, Químicos y Naturales. Universidad Pablo de Olavide, Ctra. Utrera, km 1, ES-41013, Seville, Spain.
PhD in Chemistry and professor at the University Pablo de Olavide in Seville, Spain. Her research activity focuses on synthesis and characterization of nanoparticles and nanostructures applied to biomedicine, sensors and cultural heritage.

Pilar Ortiz Calderón
mportcal@upo.es

Departamento de Sistemas Físicos, Químicos y Naturales. Universidad Pablo de Olavide, Ctra. Utrera, km 1, ES-41013, Seville, Spain. PhD in Chemistry and professor at the University Pablo de Olavide in Seville, Spain. Her research activity focuses on risk and vulnerability applied to cultural heritage, diagnosis of artwork conservation, non destructive techniques and assessment of restoration treatments.