Laser re-irradiation of palladium nanoparticles for antibacterial applications

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Summary

Bacterial resistant infections represent the second leading cause of mortality worldwide, being responsible for almost the 14% of the global deaths according to figures of 2019.

Nanoparticles of noble metals have received special attention because of their outstanding bactericidal activity, that is intrinsically linked to their physico-chemical properties, an asset that makes them an appealing alternative to combat resistant microorganisms. So as to optimize their applications within the biomedical field, it becomes relevant to develop and employ means to influence and act upon nanoparticles' physico-chemical characteristics.

In this research, palladium nanoparticles were synthesized via laser ablation. A nanosecond Nd:YVO_4 laser operating at 532 nm was employed to ablate a palladium target immersed in deionized water in order to obtain the colloidal suspension. Then, the previous suspension was passed twice through a re-irradiation system to narrow the size and size distribution of palladium nanoparticles. Bacterial assays with Staphylococcus aureus *revealed an effect of size on the bactericidal behaviour of palladium nanoparticles.*

1. Introduction and objectives

Antimicrobial resistance (AMR) was believed to be under control in the past decades, but since 2010 it has been generating more and more concern, as the inadequate use of antimicrobials has caused an increasing rate of resistance development and there is a shortage of new efficient drugs to combat these superbacteria [1].

Given the drug resistance on the rise, it is estimated that the burden of deaths from AMR could snowball to 10 million lives per year by 2050, above cancer, diabetes and traffic accidents, at a cumulative cost to global economic output of 100 trillion USD, if no action is taken. This implies that by 2050, one person would die every three seconds and each person in the world today would cost 10,000 USD. Those studies and statistics pointed to an increasing concern about antimicrobial resistance growth and spreading and convey despairing predictions for the future. For this reason, further research and development of novel treatments is required to create opportunities to combat this global health problem [2,3].

Ancient civilizations were aware of the beneficial properties of metals for health, such as silver (Ag), copper (Cu), gold (Au), etc., and thus, employed them for therapeutic purposes. Recent advances in the field of nanotechnology have grown an increasing interest in metallic nanoparticles (NPs) as a promising tool to treat infectious diseases, so that their physico-chemical properties determine to a large extent their bactericidal behaviour [4–7]. Among metallic NPs, silver NPs have received special attention due to its outstanding bactericidal activity at concentrations that are not cytotoxic to human cells [8–10]. However, several reports have already warned about the potential development of bacterial resistance to silver NPs after repeated exposure. Hence, it is of major importance to investigate on different metallic materials as effective as silver [11].

Laser ablation permits to synthesize nanomaterials without any chemical precursor or reaction that may produce any toxic by-products, which becomes determining when the objective of those nanomaterials is to be employed in biomedical environments. In addition, it presents remarkable advantages such as low cost, high productivity, good stability and a certain degree of both size and shape control, by adjusting processing parameters [12–14]. The close relationship between the properties of NPs and their size is perhaps the most important quality of this new family of materials and underlines the importance of finding ways to affect these dimensional parameters in order to optimise their use in different biomedical applications.

In this work, we designed a system to re-irradiate colloidal suspensions of NPs in order to decrease their size and narrow their size distribution. We studied the system requirements and defined its desirable performance. Then, a prototype was manufactured and used in laboratory assays with palladium NPs, to verify its desired functioning. Bactericidal assays with *Staphylococcus aureus* were performed with original and re-irradiated colloidal suspensions in order to analyse the influence of size on their antibacterial behaviour.

2. Materials and methods

2.1. System design and manufacture

Prior to any experimental procedure, it was necessary to define several requirements for the re-irradiation system. The system must ensure that the laser beam interacts in the

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same way with all the NPs that pass through it, in order to homogeneously re-irradiate all of them. In addition, it should integrate two storage tanks (one for the starting solution and another for the re-irradiated solution) with their own agitation systems, to maintain a homogeneous concentration inside and prevent nanoparticles in solution from agglomerating or depositing at the bottom of the tanks. Both storage tanks should be connected by a thin duct that carries the initial solution from its tank through the capillary, where it will be re-irradiated by the laser beam, to the remaining tank; preferably without any additional pumping element.

Once the requirements were defined, the experimental system was designed and built accordingly.

2.2. Nanoparticle synthesis

To obtain the Pd nanoparticles, a nanosecond Nd:YVO_4 laser operating at 532 nm was used to ablate a palladium foil of 99.9% of purity (Alfa Aesar, Massachusetts, USA) immersed in deionized water.

The main operational parameters of the laser are detailed in Table 1.

Table 1. *Processing conditions used for the experimental assays*

Operation frequency (kHz)	20
Maximum intensity (A)	27.7
Average output power (W)	5
Pulse width (µs)	1
Scanning speed (mm/s)	50

After the laser ablation process, the colloidal suspension was twice re-irradiated employing the prototype of the fragmentation system (see Figure 1). For the experimental assays, two capillaries of different section were employed (0.5 and 1.0 mm). The obtained samples are defined in Table 2. The re-irradiation process was carried out using the same laser source with the same processing parameters used to obtain the initial colloidal solution (Table 1).

Table 2. *Identification of the NPs produced*

Sample identification	Capillary width (mm)	Obtention
a0	---	As ablated NPs
a1	0.5	NPs after one re-irradiation
a2	0.5	NPs after two re-irradiations
b0	---	As ablated NPs
b1	1.0	NPs after one re-irradiation
b2	1.0	NPs after two re-irradiations

2.3. Nanoparticles' characterization

Transmission electron microscopy (TEM)

After the experimental assays, drops of each sample were deposited on Formvar/Carbon supported copper grids with 400 mesh of size (TedPella Inc., Redding, CA, USA) and left to dry before their analysis. Then, nanoparticle size, morphology and dispersion were characterized by transmission electron microscopy (TEM) using JEOL JEM 1010 equipment (JEOL, Akishima, Japan). Size distribution was studied on the basis of TEM micrographs, employing the software ImageJ to measure the diameter of 400 nanoparticles for each sample.

High transmission electron microscopy (HRTEM)

The palladium nanoparticles were also observed by high resolution transmission electron microscopy (HRTEM) using JEOL JEM 2010F FEG (JEOL, Akishima, Japan), so as to study their crystalline structure.

X-Ray diffraction (XRD)

Drops of each sample were also placed on glass sample holders to acquire a layer of the proper thickness to analyse via X-Ray diffraction (XRD), in order to examine the crystalline structure of nanoparticles. With that purpose, the Pananalytical X'Pert Pro X-ray diffractometer (Malvern Panalytical, Malvern, UK), using monochromated Cu-Kα radiation (λ = 1.54 Å) over the 30–130° 2θ range with step size of 0.026° was used. The XRD spectra of each sample was compared to that of the precursor material (palladium foil) and the ICDD-JCPDS database was employed to identify the crystalline phases.

UV-Vis spectroscopy

To conclude the physico-chemical characterization, the UV-VIS spectra of the samples were measured to study their stability and optical properties. The spectrophotometer HP 8452 (Hewlett Packard, Palo Alto, CA, USA) was used to perform the measurements within the range 190 – 820 nm.

Bactericidal tests

The bacterial adhesion to non-treated titanium samples and samples decorated with the nanoparticles was studied with *Staphylococcus aureus* (Colección Española de Cultivos Tipo (CECT) 435, Spain) cultured in BHI broth (Scharlab SL, Sentmenat, Spain).

Control and treated samples were immersed in ethanol and distilled water for 15min each and put in a 24-multiwell plate (Nunc, USA) with 1ml bacterial suspension at 37C for 2h. The adhered bacteria were collected seeded onto agar plates supplemented with BHI medium. The agar plates were then incubated at 37°C for 24h and the CFUs counted.

3. Results and discussion

Figure 1 presents the re-irradiation system's prototype positioned on the laser processing station.

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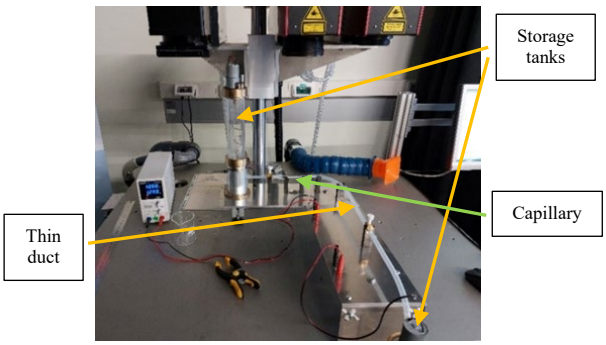

Figure 1. *System prototype positioned on the laser processing station*

The analysis of the morphology and size distribution of NPs synthesized by laser ablation and re-irradiated on the system was performed using different TEM micrographs. Figure 2 shows TEM images of Pd nanoparticles as produced and re-irradiated two times along to their corresponding size distribution histograms. Histograms were made by measuring the diameter of 400 NPs per sample.

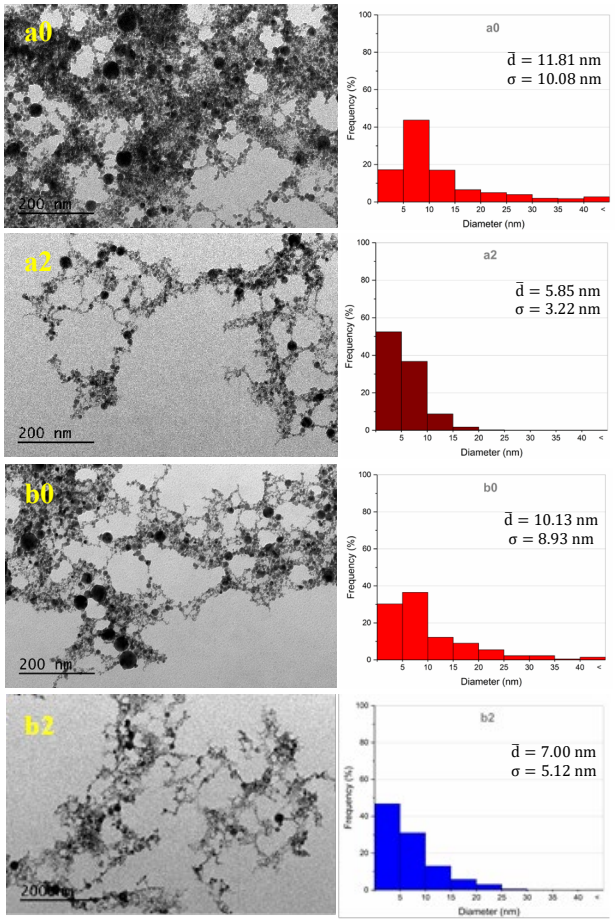

Figure 2. *TEM micrographs and size distribution of Pd NPs as obtained (a0 and b0) and re-irradiated two (a2 and b2)*

It can be observed that all Pd NPs obtained are spherical with a certain tendency to agglomeration, forming chain structures (Figure 2). The re-irradiation process does not seem to alter NPs' morphology, since all micrographs show NPs with rounded shape forming clusters. However, it indeed affects size and dispersion: the mean diameter decreases more than a 50% and a clear influence of capillary thickness can be noted.

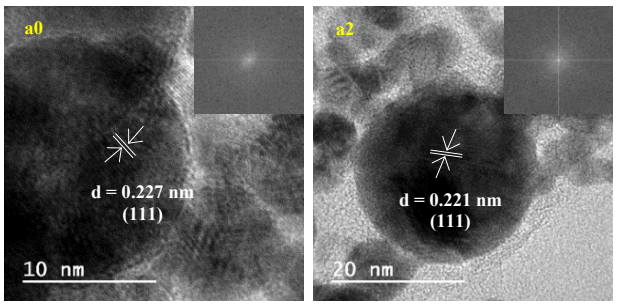

Figure 3. *HRTEM of as produced Pd NPs (a0) and re-irradiated two times (a2)*

All the particles obtained, the as-ablated as well as the re-irradiated ones, are crystalline and pure Pd. Figure 3 shows HRTEM of samples a0 and a2, with clear lattice fringes and their corresponding Fast Fourier Transform (FFT). The measured interplanar distances from the FFT correspond to the family planes of cubic Pd (JCPDS-ICDD ref. 00-005-0681). It is important to highlight that composition and crystalline structure do not seem to be significatively modified by the re-irradiation process.

The XRD analysis confirmed the elemental nature of the Pd NPs (Figure 4). All NPs are crystalline and pure palladium with characteristic diffraction peaks at 40.12° (111), 46.66° (200), 68.08° (220) and 82.09° (311).

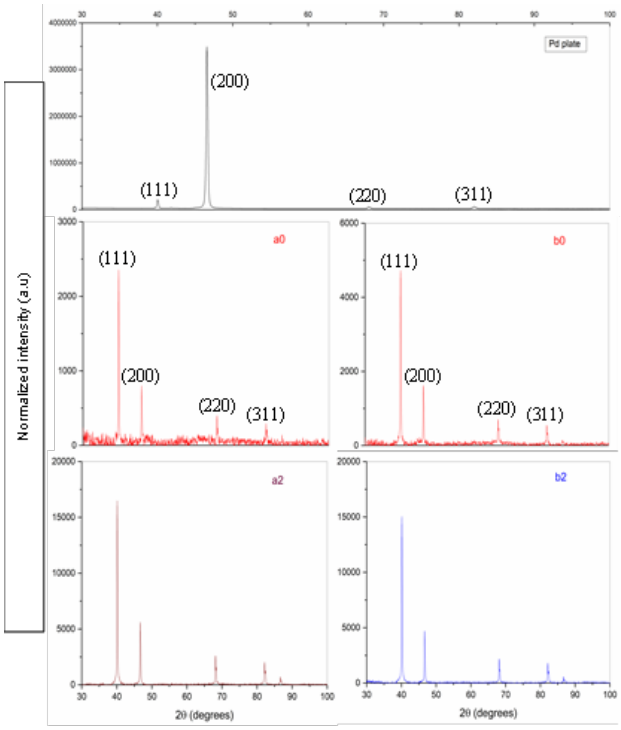

Figure 4. *XRD of the palladium plate and palladium nanoparticles: as produced (a0 and b0) and re-irradiated two times (a2 and b2)*

To conclude with the characterization, the UV-VIS spectra of the colloidal suspensions show a peak at approximately 190-200 nm (Figure 5). The narrowing in the re-irradiated solutions is indicative of a restricted size distribution, which is in agreement with TEM images.

The results from the bactericidal test are depicted in Figure 6. Regarding the graph, a smooth effect of particle size could be evidenced, as smaller NPs present higher

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antibacterial activity against *S. aureus*. Sample a2 (Table 2) exhibits the most reduced mean diameter and size dispersion, that are linked to an improved bactericidal effect. This could be due to the fact that smaller size implies higher specific surface and hence, greater reactivity and a more intense interaction with bacteria.

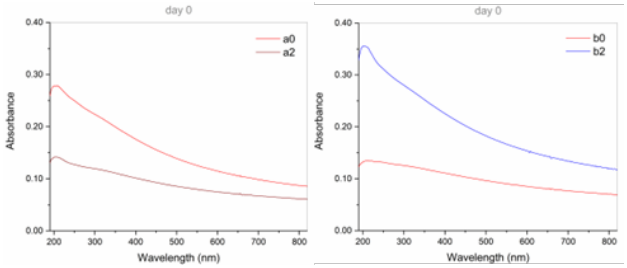

Figure 5. *UV-VIS spectra of the palladium NPs: as produced (a0 and b0) and re-irradiated two times (a2 and b2)*

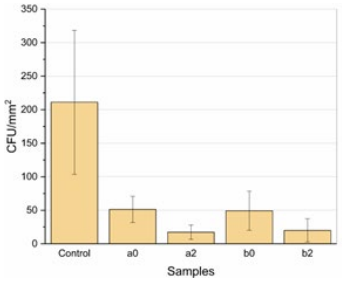

Figure 6. *Bactericidal effect of Pd NPs: as produced (a0 and b0) and re-irradiated two times (a2 and b2). Values presented as mean ± SD*

4. Conclusions

Spherical Pd NPs with crystalline structure were synthesized via laser ablation technique using a nanosecond Nd:YVO_4 laser operating at 532 nm. A fragmentation system was designed and manufactured to re-irradiate the nanoparticle suspensions in order to reduce their mean size and size dispersion. Bacterial adhesion assays with *Staphylococcus aureus* were performed to analyse the effect of particle size on antimicrobial effectiveness of Pd NPs. The results acquired seem to indicate that size plays a significant role on bactericidal activity, as smaller Pd NPs with higher specific surface present increased activity against *S. aureus*, but more tests are required to completely understand their bactericidal and cytotoxic behaviour prior to their application as therapeutic treatment in humans.

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