Gold Nanoparticles contaminated by Bacterial Endotoxin: biophysical characterization, imaging and nanotoxicology

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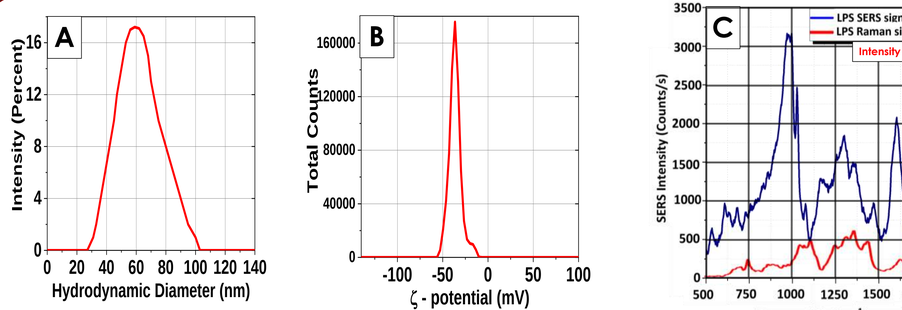
Abstract

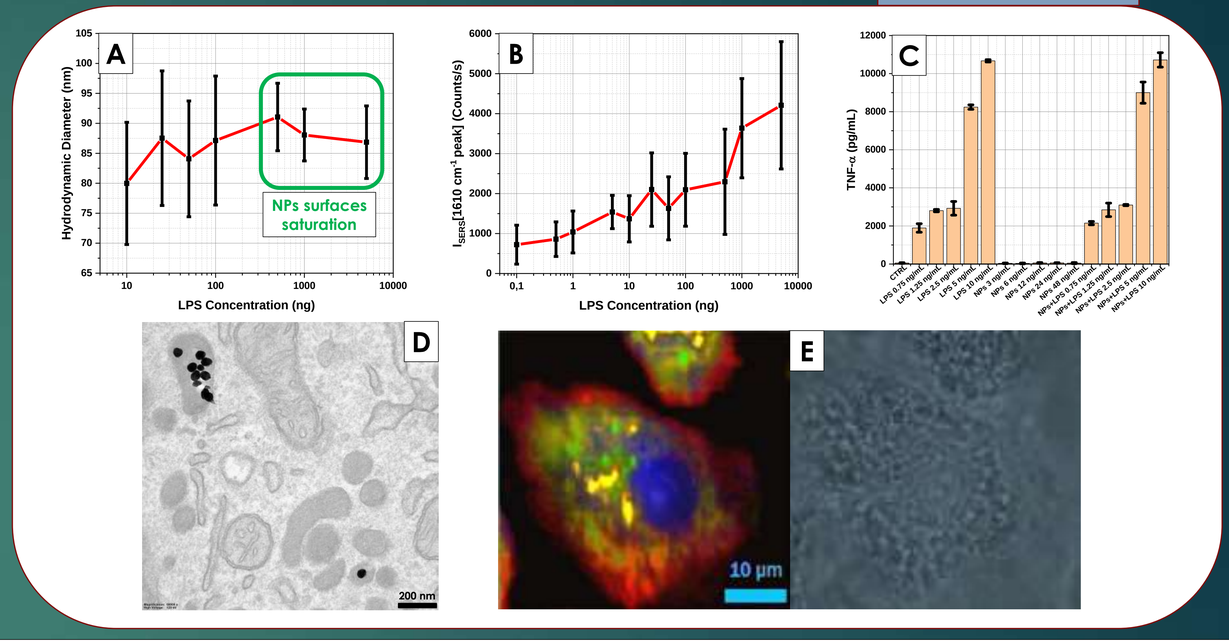
Gold nanoparticles (Au NPs) are nanodevices that can have many uses in biomedical applications but unfortunately they show in some cases nanotoxic effects on biological systems [1]. Among the different NPs effects in cells, activation of immune responses is considered a central issue for assessing health risks of AuNPs [2]. The main goal of this study is to identify and analyse the activation of the inflammatory response associated to AuNPs and/or to the presence of bacterial endotoxin (or Lipopolysaccharide, LPS) on the nanoparticles' surface. To this aim, the interaction of AuNPs with LPS is analysed, the presence of LPS molecules on AuNPs is quantified, and the interaction of AuNPs with human primary macrophages is investigated, in order to distinguish the intrinsic NPs biological effects from those induced by LPS.

Methods



Dynamic Light Scattering (DLS) was used to study hydrodynamic diameter and ζ-potential of bare and LPS coated 50 nm AuNPs (A and B) [3]. LPS Raman spectrum and its SERS signal were acquired in order to characterize LPS molecule and quantify its presence on AuNPs (C) [4]. Internalization process of AuNPs in human primary macrophages was studied with TEM and Raman imaging (D and E) [5].

Results



Hydrodynamic diameter as a function of LPS concentration (A). Variability of measurements becomes greter below 500 ng, indicating that LPS incubation doses cannot cover all 50 nm AuNPs and no saturation of NPs surfaces occurs. 1610 cm^{-1} peak SERS intensity of LPS spectra as a function of LPS incubation doses (B). TNF-α cytokine production for bare and LPS coated NPs (C). TEM and Raman imaging of 50 nm AuNPs treated macrophages (D and E).

Conclusions

DLS results indicate that a uniform LPS corona (8712 molecules) is formed around all NPs (2 µg) when incubated with doses greater than 500 ng. SERS measurements show that a good signal is obtained till incubation doses of 0.1 ng, corresponding to an amount of LPS on NPs of the order of fg. Moreover, bare NPs does not induce the production of TNF-α cytokine in treated macrophages. On the other hand, NPs imaging studies showe that NPs are localized in vesicles inside macrophages cytoplasm.

References

[1] Chen, YS., Hung, YC., Liau, I. et al. Nanoscale Res Lett (2009)4:858
[2] Boraschi, D., and A. Duschl. 2012. A.NANOTOXICOLOGY 1173 edited by B. Fadeel, Pietroiusti, and A.A. Shvedova,169–184. Amsterdam:Academic Press.
[3] Yang Li, Zhenzhen Shi, Isabella Radauer-Preiml, Ancuela Andosch, Eudald Casals, Ursula Luetz-Meindl, Macarena Cobaleda, Zhoumeng Lin, Majid Jaberi-Douraki, Paola Italiani, Jutta Horejs-Hoeck, Martin Himly, Nancy A. Monteiro-Riviere, Albert Duschl, Victor F. Puntes & Diana Boraschi (2017) Nanotoxicology, 11:9-10, 1157-1175
[4] Anna Chiara De Luca, Peter Reader-Harris, Michael Mazilu, Stefania Mariggiò, Daniela Corda, and Andrea Di Falco ACS Nano 2014 8 (3), 2575-2583
[5] Managò, S, Migliaccio, N, Terracciano, M, et al. J. Biophotonics. 2018; 11:e201700207.

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