Structural and Biological Evaluation of Heparin Reduced and Covered Gold Nanoparticles

László Szabó[a], István Sz. Tódor[a], Botond L. Simon[a], Loredana F. Leopold[b], Oana T. Marişca-Truică[a], Vasile Chiş[a], Nicolae Leopold[a]

[a]Faculty of Physics, Babeş-Bolyai University, Kogălniceanu 1, 400084 Cluj-Napoca, Romania

[b]Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Mănăştur 3-5, 400372 Cluj-Napoca, Romania

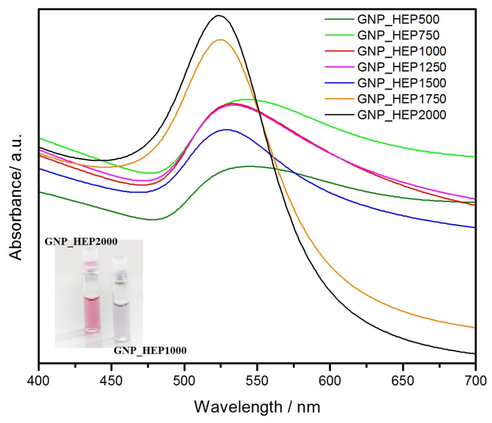
Introduction

In recent years metal nanoparticle synthesis has evolved substantially, now being possible to control their shape, size and surface chemistry. Gold nanoparticles are one of the most important types of nanoparticles with biomedical application, due to their utilization in live tissue as contrast agents, delivery vehicles, therapeutics etc [1]. Nanoparticles are known to self-assemble into larger structures during the growth processes, which are governed by a delicate balance between electrostatic repulsion and Van der Waals attraction [2]. Many nanoparticle superstructures with new properties and applications have been developed, mimicking the behavior of efficient natural machines (e.g., enzymes, proteins, biopolymers, or viruses) [3].

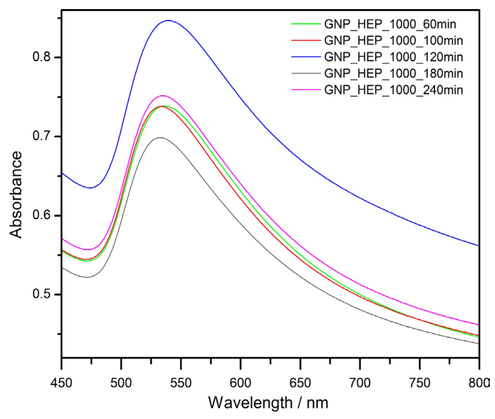
Methods

A simple method for preparing gold nanoparticles (GNP) in aqueous solution has been developed by using heparin, with different concentrations, as reducing and stabilizing agent and $HAuCl_4$ as precursor. Colloidal gold GNP were prepared by chemical reduction of $HAuCl_4$ in the presence of heparin with major modifications as described previously by Guo et al. [4]. In a 100-mL round-bottom was added 100 µL chloroauric acid solution (1 g $HAuCl_4$ / 50 mL H_2O) then, a solution of heparin (10 mg heparin/ 10 mL H_2O) was injected and stirred for about 300 minutes at 100ºC. All glassware used was cleaned in a bath of freshly prepared aqua regia solution (HCl:HNO_3 3:1) and then rinsed thoroughly with H_2O prior to use. The characterization of the heparin reduced gold nanoparticles (GNP_HEP) were carried out by using UV-Vis, IR, TEM, Microscopy imaging, SERS spectroscopy, MTT and ROS studies.

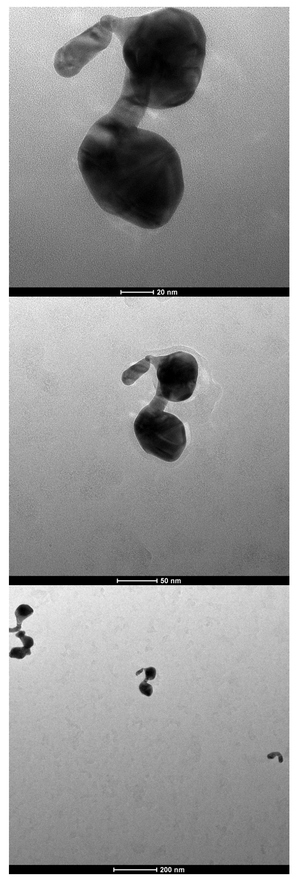
Results A: Nanoparticle characterization



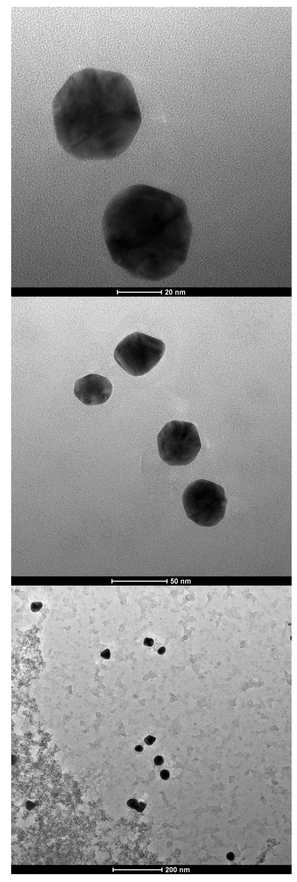
Concentration dependent UV-Vis spectra of GNP_HEP. The inset image shows a picture of the two colloids.



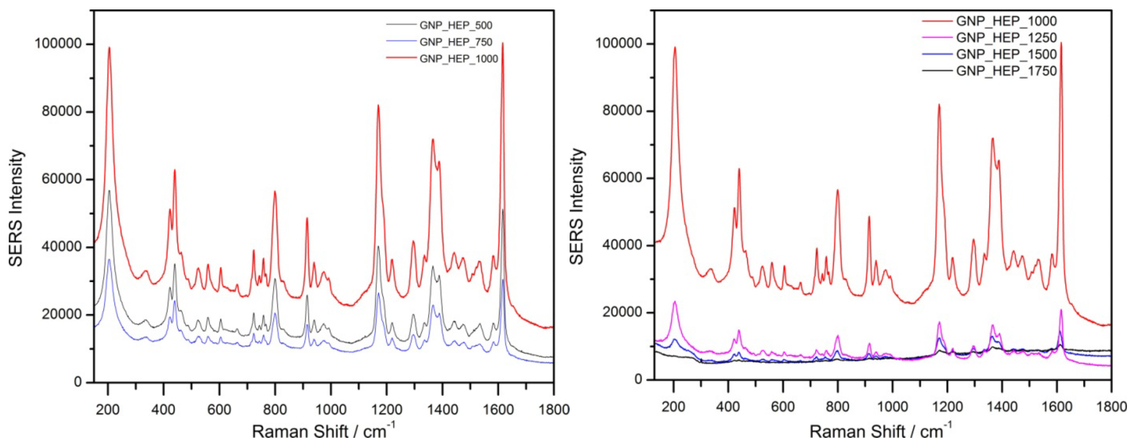
Preparation time dependent UV-Vis spectra of GNP_HEP.



TEM images of the GNP_HEP_1000 colloid.

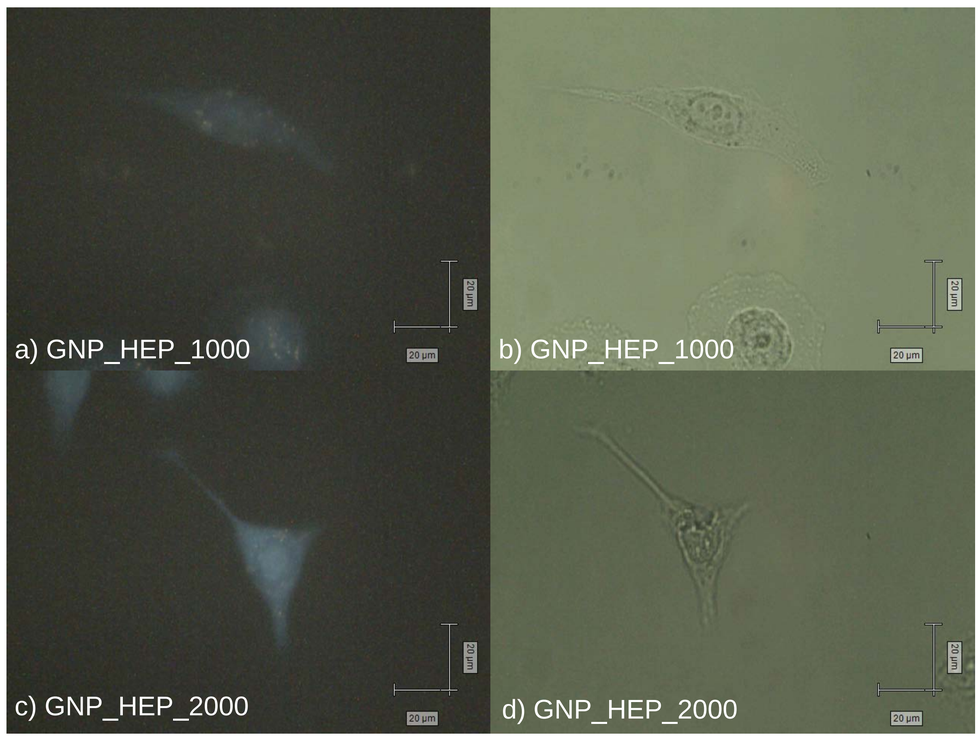


TEM images of the GNP_HEP_2000 colloid.

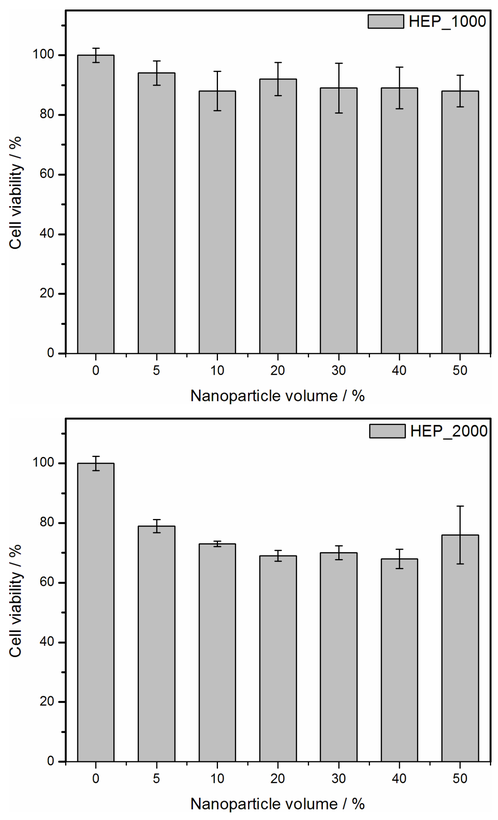


SERS spectra of Crystal Violet at 10^{-6} M concentration obtained with GNP_HEP_500/750/1000/1250/1500/1750.

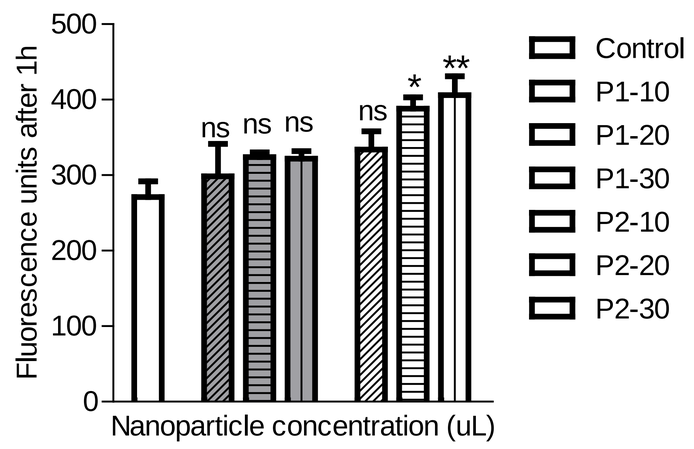
Results B: Nanoparticle cytotoxicity



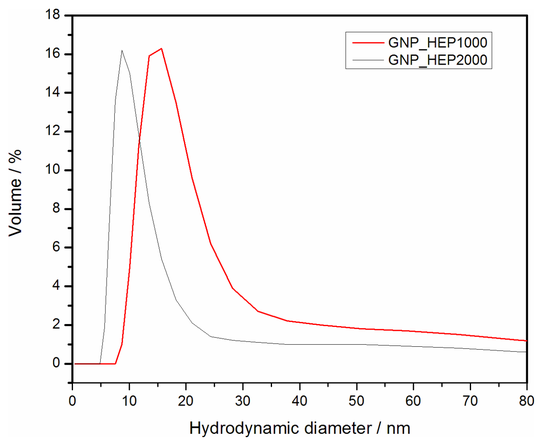
Microscopy images obtained by reflected illumination (a, c) and transmitted light microscopy images (b, d) of an A549 cell treated with nanoparticles.



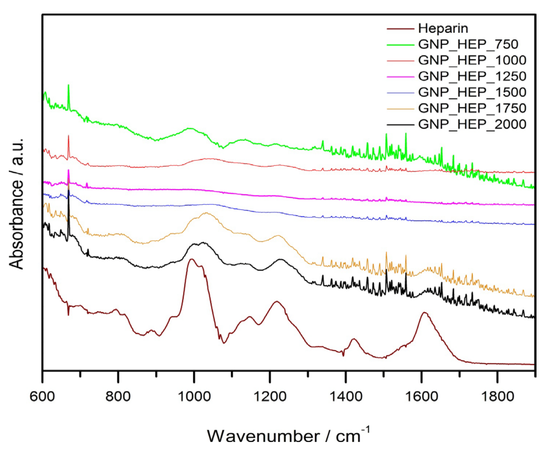
Viability test results of the A549 cell cultures treated with GNP_HEP_1000 (top) and GNP_HEP_2000 (bottom).



ROS tests of A549 cells treated with HEP_1000 (P1) and HEP_2000 (P2) at different concentrations.



DLS spectra of GNP_HEP1000 vs GNP_HEP2000.



FTIR spectra of heparin and GNP_HEP reduced at different heparin concentration.

References

[1] E. C. Dreaden, et al., Chemical Society Reviews 41, 2740-2779 (2012).
[2] Y. Xia, et al., Nature Nanotechnology 6, 580-587 (2011).
[3] B. Pelaz et al., ACS Nano 6, 8468-8483 (2012).
[4] Guo, Y. and H. Yan, Preparation and Characterization of Heparin-Stabilized Gold Nanoparticles. Journal of Carbohydrate Chemistry, 2008. 27(5): p. 309-319.

Conclusion

The UV-Vis spectra recorded at different times of reaction shows that 100 minutes is the best reaction time in order to obtain the most uniform GNP_HEP. Raman measurements revealed that GNP_HEP_1000 nanoparticles gives the most intense signals, in consequence these nanoparticles are the most suitable ones for SERS. The FTIR measurements shows that the heparin is present on the surface of the GNP_HEP. From the MTT and ROS tests it can be concluded that GNP_HEP_1000 nanoparticles are not toxic. Taking into account the above mentioned results and the fact that the heparin is binding on thrombotic tissue our GNP_HEP are promissory for surface enhanced spatial offset Raman spectroscopy (SESORS) for monitoring thrombosis.

Acknowledgements

This work was supported by CNCS-UEFISCDI, project number PN-II-RU-TE-2012-3-0227/2013.