Volume 9, Issue 2, 2019, 3894 - 3897 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

https://doi.org/10.33263/BRIAC92.894897

Original Research Article **Open Access Journal**

Received: 22.02.2019 / Revised: 03.04.2019 / Accepted: 04.04.2019 / Published on-line: 15.04.2019

Using atomic force microscopy to assess surface modification of gold nanoparticles

Irina V Safenkova [1,*], Anatoly V Zherdev [1], Boris B Dzantiev [1]

[1]A.N. Bach Institute of Biochemistry, Research Centre of Biotechnology of the Russian Academy of Sciences Leninsky prospect 33, Moscow 119071, Russia

*corresponding author e-mail address: ***irina.safenkova@gmail.com***

ABSTRACT

This paper proposed an approach for analyzing surface modification of nanoparticles based on atomic force microscopy (AFM). The work was carried out on unmodified gold nanoparticles and gold nanoparticles coated with a protein shell (a conjugate of gold nanoparticles and immunoglobulin G). The proposed analysis of surface modification of gold nanoparticles consists of the registration of force curves, which reflect the dependences of the deflection of the beam of the AFM probe when interacting with the surface from moving the cantilever in the vertical direction. The elastic modulus and the force of the interaction between the probe and the modified or unmodified surface of the nanoparticles are proposed as the parameters characterizing the modification. Significant differences were obtained between the unmodified and modified gold nanoparticles using the AFM method in the spectroscopy mode.

Keywords: *Gold nanoparticles, atomic force microscopy, protein shell, immunoglobulin G, spectroscopy mode.*

1. INTRODUCTION

Gold nanoparticles (GNPs) with plasmon resonance have various applications in nanobiotechnology and nanomedicine due to their ability to vary the spectral position and amplitude of plasmon resonance by changing the size, shape, and structure of the particles [1-4]. The results of a number of studies that have been conducted in recent years testify to the peculiarities of the biological effect of nanomaterials that distinguish them from traditional compounds [5-10]. This creates opportunities for using nanomaterials in medicine, pharmacology, and food production, and for solving environmental and agricultural problems. However, the high biological activity of technogenic nanoparticles (NPs) carries the potential risks of toxic effects for workers in nanotechnological enterprises and consumers of nanoindustry products who come into contact with nanotechnological materials and the resulting waste [11-17].

NPs usually enter the body in the form of aggregates/associates and/or with a surface modified as a result of interacting with the biological environment. Many papers have reported on the surface modification of NPs by proteins (adsorption formation of the protein corona) and other polymers of the biological matrix, *in vitro* and *in vivo* [18-23]. The presence of a protein corona can significantly affect the biocompatibility and bio-distribution of man-made NPs [24-29], including their recognition by cells in the immune system.

Assessment of surface modification of NPs is challenging. In this regard, force spectroscopy as a mode of atomic force microscopy (AFM) is a very useful tool for characterizing NPs. One of its valuable applications is to quantify the elasticity of NPs that is associated with their surface modification. In force spectroscopy experiments that investigate this topic, a sharp tip of a cantilever is indented in one NP, and then Young's modulus is defined as a grade of its elasticity, which is one of the important mechanical parameters that affect the functional properties of NPs. In their review [30], Sun et al. summarized the approaches used to detect the elasticity of NPs and the divergent elasticity values of GNPs with different surfaces. In spite of the interest in this subject, only one study by Wampler et al. [31] described the elasticity of GNPs whose surface was modified with bovine serum albumin and streptavidin.

Therefore, the present study aimed to develop an approach based on AFM for the analysis of surface modification of GNPs with a common protein of a protein corona: immunoglobulin G (IgG). The development of this type of analytical protocol is highly requested due to the wide use of GNP-IgG conjugates for analytical and therapeutic purposes.

2. MATERIALS AND METHODS

When determining the surface properties of GNPs and an aliquot of their conjugates in a solution of NPs synthesized according to the method in [32] or their conjugates (5–10 μL) synthesized according to [33], the surface of the substrate (freshly split mica) was incubated at room temperature for 20 min, excess liquid was removed with filter paper, and the samples were dried at room temperature in a calcium chloride desiccator.

The samples were scanned in the contact mode using fpC10S cantilevers (Nanotuning (Russia)), with a radius with a curvature ≤10 nm and stiffness <0.2 N/m. The samples were sequentially scanned in areas with the following dimensions: 35x35 μm^2, 10x10 μm^2, 5x5 μm^2, and 1x1 μm^2. Then, the microscope was switched to spectroscopy mode, and we obtained the force curves from the selected objects in the force coordinates (F, is the ordinate axis) and the probe (tip)-surface distances (z, is the abscissa axis). The force curve for each object was obtained in five repetitions.

Measurements were obtained using a SmartSPM AFM (Aist-NT (Russia)). The original image was subjected to mathematical processing using the Gwiddion program (Czech Metrology Institute, Brno, Czech Republic) to eliminate distortions, such as non-linear scanning, temperature drift, etc.

3. RESULTS

Images of unmodified GNPs and GNPs coated with protein molecules or subjected to other transformations obtained using the AFM method cannot be reliably distinguished by only using the scanning mode. Figure 1 and Figure 2 present images of the GNPs and their conjugates with IgG, respectively. As can be seen, the morphological features are not needed to determine the degree of modification of the initial NPs.

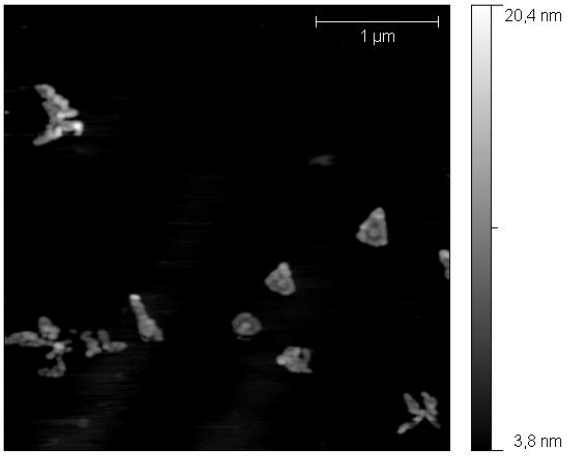

Figure 1. Images of the GNPs obtained by AFM.

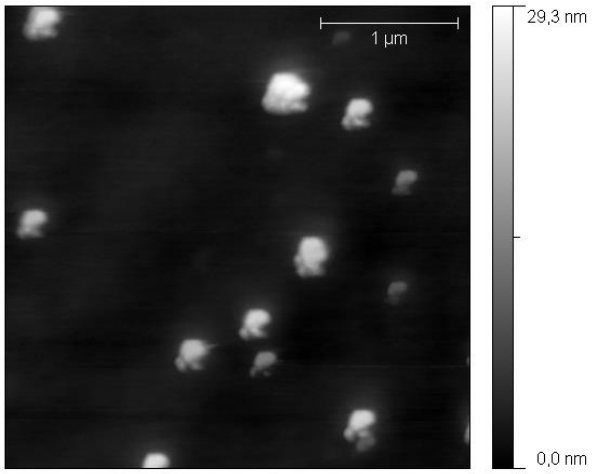

Figure 2. Images of the conjugates of GNPs with IgG obtained by AFM.

The proposed approach to characterize surface modification of GNPs is conducted by measuring the stiffness parameter (elastic modulus, Young's modulus) and the forces of the interactions between the probe and the modified or unmodified surface of the NP. AFM enables one to measure the modulus of elasticity based on the determination of the degree of deformation of the surface during its interaction with the tip of the probe of an atomic force microscope. The method consists of registering the force curves, which reflect the deflection of the flexible beam of the AFM probe when interacting with the surface, depending on the distance between them. To calculate the absolute value of Young's modulus from the force curves, the technique uses the Hertz model, which considers the interaction of a rigid hemisphere (AFM probe) and an infinite plane (surface of an NP or a modified NP).

To obtain reliable differences between unmodified and modified GNPs, we consider, in more detail, measurements in the AFM mode, in which the cantilever moves in the vertical direction. According to Hooke's law (F = kd, where F corresponds to the separation force, k to the cantilever stiffness constant, and d to the cantilever deflection), the separation force is directly proportional to the deflection of the cantilever.

When measuring the modulus of elasticity of NPs on the force curve, it is possible to determine the real distance between the probe and the sample (δ) according to Hooke's law:

$$F = kd = (z - \delta)\,, \qquad (1)$$

where F is the force of interaction between the cantilever and the sample, k is the stiffness of the cantilever, d is the deflection of the cantilever, z is the displacement of the piezo scanner vertically, and δ is the deformation of the sample in the approximation of the non-deformed probe (real distance between the tip of cantilever and the sample).

The modulus of elasticity was determined with the Hertz model, using the following formula:

$$F = \frac{4\,E}{3\cdot(1-\nu^2)} \cdot \delta^{1,5}\,\sqrt{R}\,, \qquad (2)$$

where E is the modulus of elasticity, ν is the Poisson's ratio, and R is the tip`s radius.

In this way

$$F = A\delta^{1,5}\,, \qquad (3)$$

where A is the proportionality coefficient.

By plotting the logarithm of the force versus the logarithm of the deviation, we obtain a straight line of the form Y = kX + B, where k ≈ 1.5, B = lnA. Using formula (2) we found the values of the local modulus of elasticity.

The interaction forces estimate is calculated on the basis of the force curves, that is, the dependences of the tip deflection from the movement of the cantilever in the vertical direction (Figure 3). Due to software-based signal processing methods, the force curve can be transformed into the dependence of the separation force on the displacement in the vertical direction. The appearance of a significant local minimum on the force curve of modified GNPs should be noted.

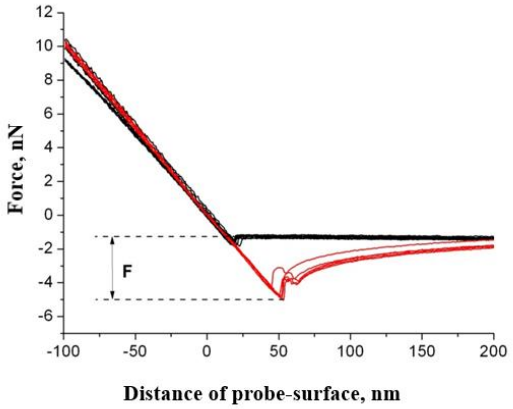

Figure 3. The dependence of the force in the Z coordinate on the distance of the probe-surface when the cantilever is brought to the surface of the unmodified GNPs (black curves) and the conjugates of GNPs with IgG (red curves).

To measure the interaction forces, a vertical projection was plotted along the ordinate axis (see the F value in Figure 3) corresponding to the segment from the local minimum on the retraction force curve to the horizontal segment on the approach curve. This vertical projection reflects the value of the forces of interaction between the probe and the modified NP surface. The degree of surface modification of NPs could be defined as the ratio of the force of the interaction between the probe and the unmodified surface of the NP to the force of the interaction between the probe and the modified surface or the ratio of the elastic modulus of the unmodified NPs to the elastic modulus of the modified NPs.

The obtained results demonstrate that the AFM is an effective tool for characterizing the surface modification of GNPs with a protein corona, thereby providing useful data about widely used GNP-IgG complexes.

4. CONCLUSIONS

The high spatial resolution of the AFM, together with the simplicity of sample preparation and the ability to work in a liquid medium, enable the developed approach to be used to study the surface properties of various modified and unmodified NPs. The obtained results allow us to conclude that assessment of surface modification of NPs using the AFM method in the spectroscopic mode seems to be a promising direction and an effective way to record the unique characteristics of NP complexes with bioorganic molecules.

5. REFERENCES

1. Alex, S.; Tiwari, A. Functionalized gold nanoparticles: synthesis, properties and applications—a review. *Journal of Nanoscience and Nanotechnology* **2015,** *15*, 1869-1894, http://dx.doi.org/10.1166/jnn.2015.9718.
2. Dykman, L.; Khlebtsov, N. Gold nanoparticles in biomedical applications: recent advances and perspectives. *Chemical Society Reviews* **2012,** *41*, 2256-2282, https://doi.org/10.1039/c1cs15166e.
3. Amendola, V.; Pilot, R.; Frasconi, M.; Marago O.M.; Iati M.A. Surface plasmon resonance in gold nanoparticles: a review. *Journal of Physics-Condensed Matter* **2017,** *29*, 48, https://doi.org/10.1088/1361-648X/aa60f3.
4. Chen, H.; Zhou K.; Zhao G.H. Gold nanoparticles: From synthesis, properties to their potential application as colorimetric sensors in food safety screening. *Trends in Food Science & Technology* **2018,** *78*, 83-94, https://doi.org/10.1016/j.tifs.2018.05.027.
5. Hassellöv, M.; Readman, J.W.; Ranville, J.F.; Tiede, K. Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. *Ecotoxicology* **2008,** *17*, 344-361, https://doi.org/10.1007/s10646-008-0225-x.
6. Tiede, K.; Boxall, A.B.; Tear, S.P.; Lewis, J.; David, H.; Hassellöv, M. Detection and characterization of engineered nanoparticles in food and the environment. *Food Additives and Contaminants* **2008,** *25*, 795-821, https://doi.org/10.1080/02652030802007553.
7. Srivastava, V.; Gusain, D.; Sharma, Y.C. Critical review on the toxicity of some widely used engineered nanoparticles. *Industrial & Engineering Chemistry Research* **2015,** *54*, 6209-6233, https://doi.org/10.1021/acs.iecr.5b01610.
8. Jeevanandam, J.; Barhoum, A.; Chan, Y.S.; Dufresne, A.; Danquah, M.K., Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology* **2018,** *9*, 1050-1074, https://doi.org/10.3762/bjnano.9.98.
9. Ajdary, M.; Moosavi, M.; Rahmati, M.; Falahati, M.; Mahboubi, M.; Mandegary, A.; Jangjoo, S.; Mohammadinejad, R.; Varma, R. Health concerns of various nanoparticles: A review of their in vitro and in vivo toxicity. *Nanomaterials* **2018,** *8*, 634, https://doi.org/10.3390/nano8090634.
10. Lee, J.H.; Cho, H.Y.; Choi H.K.; Lee J.Y.; Choi J.W. Application of gold nanoparticle to plasmonic biosensors. *International Journal of Molecular Sciences* **2018,** *19*, 2021, https://doi.org/10.3390/ijms19072021.
11. Bostrom, A.; Löfstedt, R.E. Nanotechnology risk communication past and prologue. *Risk Analysis: An International Journal* **2010,** *30*, 1645-1662, https://doi.org/10.1111/j.1539-6924.2010.01521.x.
12. Conti, J.A.; Killpack, K.; Gerritzen, G.; Huang, L.; Mircheva, M.; Delmas, M.; Harthorn, B. H.; Appelbaum, R. P.; Holden, P. A. Health and safety practices in the nanomaterials workplace: results from an international survey. *Environmental Science & Technology* **2008,** *42*, 3155-3162, https://doi.org/10.1021/es702158q.
13. Cerrillo, C.; Barandika, G.; Igartua, A.; Areitioaurtena, O.; Mendoza, G., Key challenges for nanotechnology: Standardization of ecotoxicity testing. *Journal of Environmental Science and Health, Part C* **2017,** *35*, 104-126, https://doi.org/10.1080/10590501.2017.1298361.
14. Sharma, V.K.; Filip, J.; Zboril, R.; Varma, R.S. Natural inorganic nanoparticles–formation, fate, and toxicity in the environment. *Chemical Society Reviews* **2015,** *44*, 8410-8423, https://doi.org/10.1039/c5cs00236b .
15. Sukhanova, A.; Bozrova, S.; Sokolov, P.; Berestovoy, M.; Karaulov, A.; Nabiev, I. Dependence of nanoparticle toxicity on their physical and chemical properties. *Nanoscale Research Letters* **2018,** *13*, 44, https://doi.org/10.1186/s11671-018-2457-x.
16. Wei, Y.C.; Quan, L.; Zhou, C.; Zhan, Q.Q. Factors relating to the biodistribution & clearance of nanoparticles & their effects on in vivo application. *Nanomedicine* **2018,** *13*, 1495-1512, https://doi.org/10.2217/nnm-2018-0040.
17. Meng, H.; Leong, W.; Leong, K.W., Chen, C.Y., Zhao, Y.L. Walking the line: The fate of nanomaterials at biological barriers. *Biomaterials* **2018,** *174*, 41-53, https://doi.org/10.1016/j.biomaterials.2018.04.056.
18. Aggarwal, P.; Hall, J.B.; McLeland, C.B.; Dobrovolskaia, M. A.; McNeil, S.E. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Advanced Drug Delivery Reviews* **2009,** *61*, 428-437, https://doi.org/10.1016/j.addr.2009.03.009.
19. Casals, E.; Pfaller, T.; Duschl, A.; Oostingh, G.J.; Puntes, V. Time evolution of the nanoparticle protein corona. *ACS Nano* **2010,** *4*, 3623-3632, http://dx.doi.org/10.1021/nn901372t.
20. Dobrovolskaia, M.A.; Patri, A.K.; Zheng, J.; Clogston, J.D.; Ayub, N.; Aggarwal, P.; Neun, B.W.; Hall, J.B.; McNeil, S.E. Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. *Nanomedicine: Nanotechnology, Biology and Medicine* **2009,** *5*, 106-117, https://doi.org/10.1016/j.nano.2008.08.001.
21. Pederzoli, F.; Tosi, G.; Vandelli, M.A.; Belletti, D.; Forni, F.; Ruozi, B. Protein corona and nanoparticles: how can we investigate on? *Wiley Interdisciplinary Reviews: Nanomedicine*

and Nanobiotechnology **2017,** *9*, 1467, https://doi.org/10.1002/wnan.1467.
22. Canesi, L.; Balbi, T.; Fabbri, R.; Salis, A.; Damonte, G.; Volland, M.; Blasco, J. Biomolecular coronas in invertebrate species: Implications in the environmental impact of nanoparticles. *NanoImpact* **2017,** *8*, 89-98, https://doi.org/10.1016/j.impact.2017.08.001.
23. Pareek, V.; Bhargava, A.; Bhanot, V.; Gupta, R.; Jain, N.; Panwar, J. Formation and characterization of protein corona around nanoparticles: a review. *Journal of Nanoscience and Nanotechnology* **2018,** *18*, 6653-6670, https://doi.org/10.1166/jnn.2018.15766.
24. Docter, D.; Westmeier, D.; Markiewicz, M.; Stolte, S.; Knauer, S.; Stauber, R. The nanoparticle biomolecule corona: lessons learned–challenge accepted? *Chemical Society Reviews* **2015,** *44*, 6094-6121, https://doi.org/10.1039/c5cs00217f.
25. Jain, P.; Pawar, R.; Pandey, R.; Madan, J.; Pawar, S.; Lakshmi, P.; Sudheesh, M. In-vitro in-vivo correlation (IVIVC) in nanomedicine: Is protein corona the missing link? *Biotechnology Advances* **2017,** *35*, 889-904, https://doi.org/10.1016/j.biotechadv.2017.08.003.
26. Charbgoo, F.; Nejabat, M.; Abnous, K.; Soltani, F.; Taghdisi, S.M.; Alibolandi, M.; Shier, W.T.; Steele, T.W.J.; Ramezani, M. Gold nanoparticle should understand protein corona for being a clinical nanomaterial. *Journal of Controlled Release* **2018,** *272*, 39-53, https://doi.org/10.1016/j.jconrel.2018.01.002.
27. Bros, M.; Nuhn. L.; Simon, J.; Moll, L.; Mailander, V.; Landfester, K.; Grabbe, S. The protein corona as a confounding variable of nanoparticle-mediated targeted vaccine delivery. *Frontiers in Immunology* **2018,** *9*, 1760, https://doi.org/10.3389/fimmu.2018.01760.
28. Charbgoo, F.; Nejabat, M.; Abnous, K.; Soltani, F.; Taghdisi, S.M.; Alibolandi, M.; Shier, W.T.; Steele, T.W.J.; Ramezani, M. Gold nanoparticle should understand protein corona for being a clinical nanomaterial. *Journal of Controlled Release* **2018,** 272, 39-53. https://doi.org/10.1016/j.jconrel.2018.01.002.
29. Wang, H.X.; Lin, Y.H.; Nienhaus, K.; Nienhaus, G.U. The protein corona on nanoparticles as viewed from a nanoparticle-sizing perspective. *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology* **2018,** 10, 9, https://doi.org/10.1002/wnan.1500.
30. Sun, L.; Riedel, R.; Stanciu, S.G.; Yang, F.; Hampp, N.; Xu, L.; Wu, A. Investigations on the elasticity of functional gold nanoparticles using single-molecule force spectroscopy. *Journal of Materials Chemistry B* **2018,** *6*, 2960-2971, https://doi.org/10.1039/C7TB03309E.
31. Wampler, H.P.; Ivanisevic, A. Nanoindentation of gold nanoparticles functionalized with proteins. *Micron* **2009,** *40*, 444-448, https://doi.org/10.1016/j.micron.2009.01.002.
32. Frens, G., Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature Physical Science* **1973,** *241*, 20, https://doi.org/10.1038/physci241020a0.
33. Safenkova, I.V.; Zherdev, A.V.; Dzantiev, B.B. Correlation between the composition of multivalent antibody conjugates with colloidal gold nanoparticles and their affinity. *Journal of Immunological Methods* **2010,** *357*, 17-25, https://doi.org/10.1016/j.jim.2010.03.010.

6. ACKNOWLEDGEMENTS

This research was financially supported by the Ministry of Science and High Education of the Russian Federation; state contract 14.613.21.0086 beginning on November 27, 2018; unique identifier of the project: RFMEFI61318X0086.