

Protein-corona formation on aluminum doped zinc oxide and gallium nitride nanoparticles

Journal of Applied Biomaterials &
Functional Materials
1–12
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/22808000221131881
journals.sagepub.com/home/jbf
 SAGE

Vladimir Ciobanu¹, Francesco Roncari²,
Giacomo Ceccone², Tudor Braniste¹, Jessica Ponti²,
Alessia Bogni², Giuditta Guerrini², Domenico Cassano²,
Pascal Colpo² and Ion Tiginyanu^{1,3}

Abstract

The interaction of semiconductor nanoparticles with bio-molecules attracts increasing interest of researchers, considering the reactivity of nanoparticles and the possibility to control their properties remotely giving mechanical, thermal, or electrical stimulus to the surrounding bio-environment. This work reports on a systematic comparative study of the protein-corona formation on aluminum doped zinc oxide and gallium nitride nanoparticles. Bovine serum albumin was chosen as a protein model. Dynamic light scattering, transmission electron microscopy and X-ray photoelectron spectroscopy techniques have been used to demonstrate the formation of protein-corona as well as the stability of the colloidal suspension given by BSA, which also works as a surfactant. The protein adsorption on the NPs surface studied by Bradford Assay showed the dependence on the quantity of proteins adsorbed to the available sites on the NPs surface, thus the saturation was observed at ratio higher than 5:1 (NPs:Proteins) in case of ZnO, these correlating with DLS results. Moreover, the kinetics of the proteins showed a relatively fast adsorption on the NPs surface with a saturation curve after about 25 min. GaN NPs, however, showed a very small amount of proteins adsorbed on the surface, a change in the hydrodynamic size being not observable with DLS technique or differential centrifugal sedimentation. The Circular Dichroism analysis suggests a drastic structural change in the secondary structure of the BSA after attaching on the NPs surface. The ZnO nanoparticles adsorb a protein-corona, which does not protect them against dissolution, and in consequence, the material proved to be highly toxic for Human keratinocyte cell line (HaCaT) at concentration above 25 µg/mL. In contrast, the GaN nanoparticles which do not adsorb a protein-corona, show no toxicity signs for HaCaT cells at concentration as high as 50 µg/mL, exhibiting much lower concentration of ions leakage in the culture medium as compared to ZnO nanoparticles.

Keywords

Protein-corona, nanoparticles, ZnO, GaN, cell viability

Date received: 8 June 2022; revised: 11 September 2022; accepted: 24 September 2022



References

1. Pino PD, Pelaz B, Zhang Q, Maffre P, Nienhaus GU and Parak WJ. Protein corona formation around nanoparticles – from the past to the future. *Mater Horiz* 2014; 1: 301–313.
2. Cho EC, Zhang Q and Xia Y. The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles. *Nat Nanotechnol* 2011; 6: 385–391.
3. Berg JM, Romoser A, Banerjee N, Zebda R and Sayes CM. The relationship between pH and zeta potential of ~ 30 nm metal oxide nanoparticle suspensions relevant to *in vitro* toxicological evaluations. *Nanotoxicology* 2009; 3: 276–283.
4. Kopac T. Protein corona, understanding the nanoparticle-protein interactions and future perspectives: A critical review. *Int J Biol Macromol* 2021; 169: 290–301.
5. Guo L, Feng Z, Cai L, et al. Effects of a protein-corona on the cellular uptake of ferroferric oxide nanoparticles. *J Nanosci Nanotechnol* 2016; 16: 7125–7128.
6. Salvati A, Pitek AS, Monopoli MP, et al. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat Nanotechnol* 2013; 8: 137–143.

7. Mazzolini J, Weber RJ, Chen H-S, et al. Protein Corona modulates uptake and toxicity of nanoceria via clathrin-mediated endocytosis. *Biol Bull* 2016; 231: 40–60.
8. Tenzer S, Docter D, Kuharev J, et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat Nanotechnol* 2013; 8: 772–781.
9. Kopac T and Bozgeyik K. Equilibrium, kinetics, and thermodynamics of bovine serum albumin adsorption on single-walled carbon nanotubes. *Chem Eng Commun* 2016; 203: 1198–1206.
10. Bozgeyik K and Kopac T. Adsorption of bovine serum albumin onto metal oxides: adsorption equilibrium and kinetics onto alumina and zirconia. *Int J Chem Reactor Eng* 2010; 8: 1–26.
11. Cedervall T, Lynch I, Lindman S, et al. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc Natl Acad Sci U S A* 2007; 104: 2050–2055.
12. Saha P and Kou JH. Effect of bovine serum albumin on drug permeability estimation across caco-2 monolayers. *Eur J Pharm Biopharm* 2002; 54: 319–324.
13. Yu J, Kim HJ, Go MR, Bae SH and Choi SJ. ZnO interactions with biomatrices: effect of particle size on zno-protein corona. *Nanomater* 2017; 7: 377.
14. Tomak A, Yilancioglu B, Winkler D and Karakus CO. Protein corona formation on silver nanoparticles under different conditions. *Colloids Surf A Physicochem Eng Asp* 2022; 651: 129666.
15. Giau V-V, Park Y-H, Shim K-H, Son SW and An SSA. Dynamic changes of protein corona compositions on the surface of zinc oxide nanoparticle in cell culture media. *Front Chem Sci Eng* 2019; 13: 90–97.
16. Chen H, Song Y, Cheng X and Zhang H. Self-powered electronic skin based on the triboelectric generator. *Nano Energy* 2019; 56: 252–268.
17. Pu X, Liu M, Chen X, et al. Ultrastretchable, transparent triboelectric nanogenerator as electronic skin for biomechanical energy harvesting and tactile sensing. *Sci Adv* 2017; 3: e1700015.
18. Peng X, Dong K, Ye C, et al. A breathable, biodegradable, antibacterial, and self-powered electronic skin based on all-nanofiber triboelectric nanogenerators. *Sci Adv* 2020; 6: eaba9624.
19. Jain A, Tiwari A, Verma A and Jain SK. Ultrasound-based triggered drug delivery to tumors. *Drug Deliv Transl Res* 2018; 8: 150–164.
20. Gao W, Chan JM and Farokhzad OC. PH-Responsive nanoparticles for drug delivery. *Mol Pharm* 2010; 7: 1913–1920.
21. Chen C-R and Young T-H. Neurons cultured on GaN and is associated with synapsin I and MAP2 expression. *Biomed Eng Appl Basis Commun* 2008; 20: 75–82.
22. Ito T, Forman SM, Cao C, et al. Self-assembled monolayers of alkylphosphonic acid on GaN substrates. *Langmuir* 2008; 24: 6630–6635.
23. El Kacimi A, Pauliac-Vaujour E and Eymery J. Flexible capacitive piezoelectric sensor with vertically aligned ultralong GaN Wires. *ACS Appl Mater Interfaces* 2018; 10: 4794–4800.
24. Chaturvedi N, Chowdhury R, Mishra S, et al. GaN HEMT based biosensor for the detection of breast cancer marker (C-erbB2). *Semicond Sci Technol* 2021; 36: 045018.
25. Pal P, Pratap Y, Gupta M and Kabra S. Open gate AlGaIn/GaN HEMT biosensor: Sensitivity analysis and optimization. *Superlattices Microstruct* 2021; 156: 106968.
26. Kachhawa P, Mishra S, Jain AK, et al. Antigen-antibody interaction-based GaN HEMT biosensor for C3G detection. *IEEE Sens J* 2022; 22: 6256–6262.
27. Braniste T, Tiginyanu I, Horvath T, et al. Targeting endothelial cells with multifunctional GaN/Fe nanoparticles. *Nanoscale Res Lett* 2017; 12: 486.
28. Braniste T, Tiginyanu I, Horvath T, et al. Viability and proliferation of endothelial cells upon exposure to GaN nanoparticles. *Beilstein J Nanotechnol* 2016; 7: 1330–1337.
29. Fan Z and Lu JG. Zinc oxide nanostructures: synthesis and properties. *J Nanosci Nanotechnol* 2005; 5: 1561–1573.
30. Ridhuan NS, Razak KA, Lockman Z and Abdul Aziz A. Structural and morphology of ZnO nanorods synthesized using ZnO seeded growth hydrothermal method and its properties as UV sensing. *PLoS One* 2012; 7: e50405.
31. Gao PX, Ding Y, Mai W, Hughes WL, Lao C and Wang ZL. Conversion of zinc oxide nanobelts into superlattice-structured nanohelices. *Science* 2005; 309: 1700–1704.
32. Kong XY, Ding Y, Yang R and Wang ZL. Single-crystal nanorings formed by epitaxial self-coiling of polar nanobelts. *Science* 2004; 303: 1348–1351.
33. Feng W, Chen J and Hou CY. Growth and characterization of ZnO needles. *Appl Nanosci* 2014; 4: 15–18.
34. Wang L, Chen K and Dong L. Synthesis of exotic zigzag ZnO nanoribbons and their optical, electrical properties. *J Phys Chem C* 2010; 114: 17358–17361.
35. Madlol RAA. Structural and optical properties of ZnO nanotube synthesis via novel method. *Results Phys* 2017; 7: 1498–1503.
36. Le Pivert M, Poupart R, Capochichi-Gnambodoe M, Martin N and Leprince-Wang Y. Direct growth of ZnO nanowires on civil engineering materials: smart materials for supported photodegradation. *Microsyst Nanoeng* 2019; 5: 57.
37. Denchitcharoen S, Siriphongsapak N and Limsuwan P. Growth of ZnO nanosheets by hydrothermal method on ZnO seed layer coated by spin-coating technique. *Mater Today Proc* 2017; 4: 6146–6152.
38. Mishra YK and Adelung R. ZnO tetrapod materials for functional applications. *Mater Today* 2018; 21: 631–651.
39. Sirelkhatim A, Mahmud S, Seeni A, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Lett* 2015; 7: 219–242.
40. Colon G, Ward BC and Webster TJ. Increased osteoblast and decreased Staphylococcus epidermidis functions on nanophase ZnO and TiO₂. *J Biomed Mater Res – Part A* 2006; 78A: 595–604.
41. Gupta J, Bhargava P and Bahadur D. Fluorescent ZnO for imaging and induction of DNA fragmentation and ROS-mediated apoptosis in cancer cells. *J Mater Chem B* 2015; 3: 1968–1978.
42. Martínez-Carmona M, Gun'ko Y and Vallet-Regí M. ZnO nanostructures for drug delivery and theranostic applications. *Nanomater* 2018; 8: 268.
43. Barth A. Infrared spectroscopy of proteins. *Biochimica et Biophysica Acta – Bioenergetics* 2007; 1767: 1073–1101.
44. Marmorato P, Ceccone G, Gianoncelli A, et al. Cellular distribution and degradation of cobalt ferrite nanoparticles in

- Balb/3T3 mouse fibroblasts. *Toxicol Lett* 2011; 207: 128–136.
45. Akhtar MJ, Alhadlaq HA, Alshamsan A, Majeed Khan MA and Ahamed M. Aluminum doping tunes band gap energy level as well as oxidative stress-mediated cytotoxicity of ZnO nanoparticles in MCF-7 cells. *Sci Rep* 2015; 5: 13876.
 46. Valsesia A, Desmet C, Ojea-Jiménez I, et al. Direct quantification of nanoparticle surface hydrophobicity. *Commun Chem* 2018; 1: 53.
 47. Boström M, Deniz V, Franks GV and Ninham BW. Extended DLVO theory: electrostatic and non-electrostatic forces in oxide suspensions. *Adv Colloid Interface Sci* 2006; 123-126: 5–15.
 48. Sathanikan A, Ceccone G, Bañuls-Ciscar J, et al. A bioinspired approach to fabricate fluorescent nanotubes with strong water adhesion by soft template electropolymerization and post-grafting. *J Colloid Interface Sci* 2022; 606: 236–247.
 49. Sreerama N and Woody RW. Estimation of protein secondary structure from circular dichroism spectra: Comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal Biochem* 2000; 287: 252–260.
 50. Whitmore L and Wallace BA. Protein secondary structure analyses from circular dichroism spectroscopy: methods and reference databases. *Biopolymers* 2008; 89: 392–400.
 51. Broggi F, Ponti J, Giudetti G, et al. Silver nanoparticles induce cytotoxicity, but not cell transformation or genotoxicity on Balb3T3 mouse fibroblasts. *BioNanoMaterials* 2013; 14: 49–60.
 52. Jeyachandran YL, Mielczarski E, Rai B and Mielczarski JA. Quantitative and qualitative evaluation of adsorption/desorption of bovine serum albumin on hydrophilic and hydrophobic surfaces. *Langmuir* 2009; 25: 11614–11620.
 53. Yu Q, Zhao L, Guo C, Yan B and Su G. Regulating protein corona formation and dynamic protein exchange by controlling nanoparticle hydrophobicity. *Front Bioeng Biotechnol* 2020; 8: 210.
 54. Deng ZJ, Liang M, Toth I, Monteiro MJ and Minchin RF. Molecular interaction of poly(acrylic acid) gold nanoparticles with human fibrinogen. *ACS Nano* 2012; 6: 8962–8969.
 55. Kendall M, Ding P and Kendall K. Particle and nanoparticle interactions with fibrinogen: the importance of aggregation in nanotoxicology. *Nanotoxicology* 2011; 5: 55–65.
 56. Tougaard S. Practical guide to the use of backgrounds in quantitative XPS. *J Vac Sci Technol A* 2021; 39: 011201.
 57. Shard AG. A straightforward method for interpreting XPS data from core-shell nanoparticles. *J Phys Chem C* 2012; 116: 16806–16813.
 58. Vauche L, Chanuel A, Martinez E, et al. Study of an Al₂O₃/GaN interface for normally off MOS-Channel high-electron-mobility transistors using XPS characterization: the impact of wet surface treatment on threshold voltage V_{TH}. *ACS Appl Electron Mater* 2021; 3: 1170–1177.
 59. Thakur V and Shivaprasad SM. Electronic structure of GaN nanowall network analysed by XPS. *Appl Surf Sci* 2015; 327: 389–393.
 60. Jung W-S. Reaction intermediate(s) in the conversion of β-gallium oxide to gallium nitride under a flow of ammonia. *Mater Lett* 2002; 57: 110–114.
 61. Balkaş CM and Davis RF. Synthesis routes and characterization of high-purity, single-phase gallium nitride powders. *J Am Ceram Soc* 1996; 79: 2309–2312.
 62. Lynch I and Dawson KA. Protein-nanoparticle interactions. *Nano Today* 2008; 3: 40–47.
 63. Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Bombelli FB and Laurent S. Protein-nanoparticle interactions: opportunities and challenges. *Chem Rev* 2011; 111: 5610–5637.
 64. Reed RB, Ladner DA, Higgins CP, Westerhoff P and Ranville JF. Solubility of nano-zinc oxide in environmentally and biologically important matrices. *Environ Toxicol Chem* 2012; 31: 93–99.
 65. Avramescu M-L, Chénier M, Palaniyandi S and Rasmussen PE. Dissolution behavior of metal oxide nanomaterials in cell culture medium versus distilled water. *J Nanopart Res* 2020; 22: 222.
 66. Liu Z, Lv X, Xu L, et al. Zinc oxide nanoparticles effectively regulate autophagic cell death by activating autophagosome formation and interfering with their maturation. *Part Fibre Toxicol* 2020; 17: 46.
 67. Yin H, Casey PS, McCall MJ and Fenech M. Effects of surface chemistry on cytotoxicity, genotoxicity, and the generation of reactive oxygen species induced by ZnO nanoparticles. *Langmuir* 2010; 26: 15399–15408.
 68. Tantra R, Tompkins J and Quincey P. Characterisation of the de-agglomeration effects of bovine serum albumin on nanoparticles in aqueous suspension. *Colloids Surf B Biointerfaces* 2010; 75: 275–281.
 69. Chevallet M, Gallet B, Fuchs A, et al. Metal homeostasis disruption and mitochondrial dysfunction in hepatocytes exposed to sub-toxic doses of zinc oxide nanoparticles. *Nanoscale* 2016; 8: 18495–18506.
 70. Zhang L, Jiang Y, Ding Y, et al. Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against E. Coli. *J Nanopart Res* 2010; 12: 1625–1636.
 71. Jewett SA, Makowski MS, Andrews B, Manfra MJ and Ivanisevic A. Gallium nitride is biocompatible and non-toxic before and after functionalization with peptides. *Acta Biomater* 2012; 8: 728–733.
 72. Pearton SJ, Shul RJ and Ren F. A review of dry etching of GaN and related materials. *MRS Internet J Nitride Semicond Res* 2000; 5: 11.
 73. Cypriyana P J J, S S, Angalene J LA, et al. Overview on toxicity of nanoparticles, its mechanism, models used in toxicity studies and disposal methods – A review. *Biocatal Agric Biotechnol* 2021; 36: 102117.