Assessment of Nanomaterials' Hemotoxicity

Subjects: Physiology

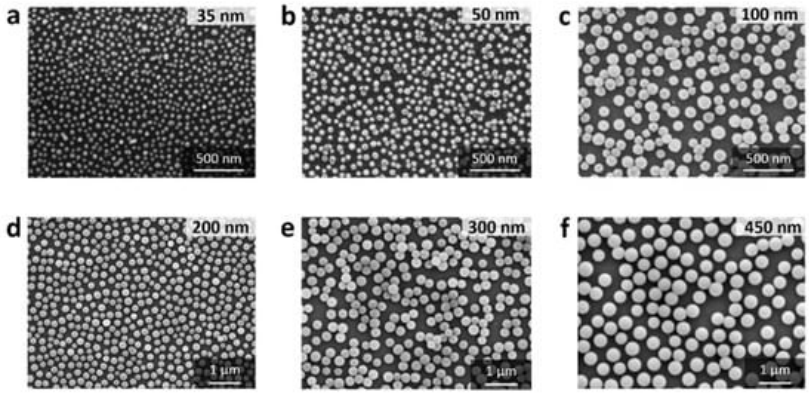
Contributor: Saul Yedgar, Gregory Barshtein, Alexander Gural

The potential use of nanomaterials in medicine offers opportunities for novel therapeutic approaches to treating complex disorders. For that reason, a new branch of science, named nanotoxicology, which aims to study the dangerous effects of nanomaterials on human health and on the environment, has recently emerged. However, the toxicity and risk associated with nanomaterials are unclear or not completely understood. The development of an adequate experimental strategy for assessing the toxicity of nanomaterials may include a rapid/express method that will reliably, quickly, and cheaply make an initial assessment. One possibility is the characterization of the hemocompatibility of nanomaterials, which includes their hemolytic activity as a marker.

Keywords: nanomaterials ; nanoparticles ; red blood cells ; hemocompatibility

1. Introduction

Engineered man-made nanomaterials have several applications in the field of biomedicine for diagnosis [1], drug delivery [2], and therapeutics [3]. The International Organization for Standardization defines nanomaterials as structures with a size range from 1 to 100 nm in one, two, or three dimensions [4]. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) [5][6] are the primary tools for the visualization of nanomaterial shapes (as illustrated in **Figure 1**). An extensive library of images of various nanomaterials has been collected [7][8][9][10][11].



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	35 nm	50 nm	100 nm	200 nm	300 nm	450 nm
Mean diameter (nm)	35	50	100	200	280	450
Standard deviation (± nm)	8	7	20	30	30	20
Particle surface density (nb/10 µm²)	2350	1600	500	140	70	35

Figure 1. The nanoparticle-based monolayers. (**a**–**f**), scanning electron microscopy images of the different fluorescent silica nanoparticles (NPs) monolayers, constructed with 35 nm, 50 nm, 100 nm, 200 nm, 300, and 450 nm NPs, respectively. (**g**) Table of the mean sizes, standard deviation, and NPs surface density (number of particles per 10 µm^2) corresponding to each NP size (all these data were obtained using ImageJ with manual thresholding). "Reproduced from [12]".

Nanomaterials are drawing increasing interest from many branches of medical practices and research [6]. Their use in medical devices or as drug carriers offers opportunities for novel therapeutic approaches to treat complex disorders such as malignant, inflammatory, and neurodegenerative diseases [13][14][15].

Humans may be exposed to nanomaterials through inhalation (respiratory tract), skin contact, ingestion, or intravenous (IV) injection. The tiny size of nanomaterials allows for them to pass more easily through cell membranes [16][17]. Moreover, some nanomaterials are readily distributed throughout the body, where they are deposited in the mitochondria of the target organs and may trigger tissue injury [16]. Possible pathways for nanoparticle uptake and intracellular transport routes have been extensively discussed in the literature, and several recent reviews are hereby recommended [18][19].

Despite the advantages offered by nanotechnology, the potential risk of intended and unintended human exposure to nanomaterials is increasing as nanotechnology develops. Novel nanomaterials are currently widely used without thoroughly assessing their potential health risks. The knowledge regarding their toxic potential is still limited, without appropriate regulatory measures being implemented [4][20][21].

Early studies on asbestos and man-made nanomaterials, such as diesel exhausts, have shown that they can accumulate in the human body, especially after daily exposure, such as in occupational settings. Long-term and short-term toxicity to humans and animals caused by nanomaterials has already become a serious concern. Therefore, a new branch of science, named nanotoxicology [22][23], has emerged, aiming to study nanomaterials' hazardous effects on human health and on the environment.

In many cases, novel nanoparticles (NPs) are widely manipulated without thoroughly assessing their potential health risks. The broad range of composition and physicochemical properties of NPs (colloidal stability, purity, inertness, size, shape, charge, etc.) make them ubiquitous and determine their interactions with other biological materials and the extent of their toxicity [24][25]. As with regular particles, the NP surface forms the point of contact with cells. Therefore, surface area and surface chemistry are essential determinants of NPs toxicity [26]. The geometry of NPs, determining their effective surface area, affects not only the interaction between NPs and plasma proteins but also the mechanism and degree of cellular absorption and, consequently, the potential toxicity of NPs [27].

Several approaches can be used to assess NP toxicity; these include epidemiological studies, human clinical studies, animal models, and in vitro models [28][29][30][31][32][33].

Whatever their use, source, and route of exposure (whether oral, respiratory, or dermal), NPs can enter the bloodstream. Several studies have reported that, due to their small size, free NPs can penetrate the alveolar lining [34][35][36], cause inflammatory reactions, and subsequently enter the bloodstream [37]. The circulation then distributes such NPs throughout the body, allowing their penetration into various organs, where they are partially metabolized, excreted, or retained. Moreover, in the bloodstream itself, the NPs interact with various blood cells [27], especially red blood cells (RBCs), the most abundant cellular component in circulation. The exposure of RBCs to NPs leads to various biochemical/biophysical and morphological changes that can significantly affect their functionality [38][39].

Under in vivo conditions (in the bloodstream), the contact between an NP and an RBC occurs in plasma, where all its components (proteins, hormones, vitamins, sugars, and inorganic ions) can affect this interaction. The effect of proteins is the most studied of all the plasma components. It is convincingly documented that the particle's surface is covered with a corona formed by adsorbed proteins in the plasma [40]. However, most publications on this subject describe NP–RBC interaction occurring in a buffer.

2. Methods for Assessment of Nanomaterials' Hemotoxicity

Nano-toxicology is a fast-developing area of nanoscience and nanotechnology. Current studies on the toxic effects of NPs, aiming to identifying the mechanisms of their harmful effects, are carried out in cell culture and animal models [41][42][43][44][45][46][47][48][49][50].

The toxicity of NPPS has received special attention [50][51][52][53]. These particles can be easily synthesized in a wide range of sizes, and their surfaces can be given different functionality [44]. Thus, they are ideally suited as a model for studying the effect of particle surface characteristics on various biological parameters both in vitro and in vivo. Sarma and colleagues [41] have analyzed the cytotoxic and genotoxic potential of NPPS on human peripheral lymphocytes (in vitro), while Loos et al. [44] have summarized information regarding the effect of functionalized (positively and negatively charged) NPPS on macrophages and THP-1 cells (in vitro). These studies indicate that while polystyrene is non-toxic, functionalized nanoparticles may behave differently than bulk material, and surface chemistry plays a critical role in determining the effect of NPPS on various cells.

The toxicity of NPPS was also analyzed in vivo in animal models [47][48][49][50]. Fan et al. [48] observed the accumulation of fluorescent NPPS in various organs of mice after oral ingestion, including in the liver, kidney, spleen, and pancreas. The main mechanism of damage to the internal organs was the impairment of liver function and lipid metabolism. Yasin and colleagues also identified the striking hepatoxicity of NPPS (in a dose-dependent manner) [50] in rats. In addition, a recent in vivo study showed that PSNPs induced reproductive toxicity [49] in mice, caused fetal growth restriction, and significantly impaired cholesterol metabolism in both the mice's placenta and the fetus [47].

However, the toxicity and risk associated with the use of NPs still need to be understood in their entirety [54]. The development of an adequate experimental strategy for estimating NPs' toxicity should include the choice between in vitro (cell lines) and in vivo (animal models) methods or a combination of both, as both methods have advantages and disadvantages. The NP toxic effects on individual cell components and tissues are more accessible for in vitro analysis, while in vivo models enable the assessment of NP toxicity for individual organs or the whole organism [51]. It seems more logical to first test NP toxicity on cells, and if toxic effects are clearly demonstrated, this may spare the need for animal testing, in accordance with the global trend of reducing the number of animal experiments [55][56].

The rapid growth of nanomedicine and the development of more and more new NPs make in vivo toxicity tests undesirable on both ethical and financial grounds, creating an urgent need to develop in vitro cell-based assays that accurately predict in vivo toxicity and facilitate safe nanotechnology.

Of all the cell types [52] that can be used to assess the toxicity of nanomaterials, the choice of RBC as a target cell seems to be the most useful. As noted above, irrespective of their use, source, and route of exposure, NPs enter the bloodstream and interact with RBCs, the major cellular component in the circulation (4–5 million RBCs per 1 µL of blood), producing a negative effect on their functionality. As RBCs are well characterized, accessible, and easy to manipulate, they make an excellent candidate for being the target cells for nanotoxicity assessment.

Numerous studies have examined the NP-RBC interaction, focusing on the hemolytic potential of NPs [53][57], suggesting that this is the critical test of NP safety [58][59]. Although hemolysis tests have been conducted with various NPs, comparing results across studies is difficult due to the variability of protocols implemented for particle characterization and hemolysis testing [60].

The American Society for Testing and Materials (ASTM) published (2008) a standard test protocol for the assessment of NPs' hemolytic properties [59], which determines the percentage of hemoglobin (Hb) released after NP-RBC interaction. The hemolytic assay has proven to be a promising test for surveying nanomaterial toxicity [61] due to its low cost, good reproducibility, and quick results [62]. To date, hemolytic activity has even been demonstrated with therapeutic NPs in vitro [63][64][65] and in vivo [66][67], indicating the potential adverse effects of NPs, which may limit their applications in nanomedicine.

Cho et al. [68] studied the nanotoxicity of a panel of NPs (CeO_2, TiO_2, carbon black, SiO_2, NiO, Co_3O_4, Cr_2O_3, CuO, and ZnO). The authors compared the acute lung inflammogenicity in a rat model with in vitro toxicity. For in vitro testing, eight different cell-based assays were used, including epithelial cells, monocytic/macrophage cells, human erythrocytes, and combined culture. Cytotoxicity in differentiated peripheral blood mononuclear cells was the most accurate, demonstrating 89% accuracy and 11% false negative results in predicting acute pulmonary inflammation. However, only hemolysis tests demonstrated a 100% match with lung inflammation at all NP concentrations. Other in vitro cellular assays showed a weaker correlation with in vivo inflammatory activity.

An analysis of the related literature supports the finding that NP-induced hemolytic activity can assess in vivo NP toxicity and has been proposed as a critical test in determining NP hemocompatibility [58][59][62][69]. However, despite the attempts to develop a unified protocol to determine NPs' hemolytic activity, the measurement conditions used by various research groups still differ significantly [60].

For a universal protocol, it is necessary to consider that forming a protein corona around NP inhibits its effective hemolytic activity. In addition, the interaction between a red cell and a nanoparticle in the bloodstream occurs under flow-induced mechanical stress, which can cause RBC deformation [70] and stimulate NP hemolytic activity [71]. Thus, it would be appropriate to test NP hemocompatibility under mechanical stress conditions in a medium supplemented by plasma proteins or in the plasma itself (and not in a buffer, as is customary in many laboratories).

The ability of an NP to change RBC properties can be expressed as an alteration in its functionality and, in its extreme form, as the destruction of the cell [38][72][73]. Therefore, other properties of red cells, such as their aggregability, deformability, and adhesion to EC, should be considered alternative markers to NP hemolytic activity [38][72][73].

All of the mentioned studies demonstrate the protective role of the protein corona formed on the nanomaterial's surface, improving the NP hemocompatibility and providing promising options for the design of therapeutic nanomaterials without prohibitive toxic effects.

Thus, it can be summarized that the NPs' characteristics and the plasma composition are the dominant factors determining the NPs' hemocompatibility. Additional factors that can affect the NP hemolytic ability inclue the properties of

the RBCs themselves and the presence of mechanical stress (**Figure 2**). For these reasons, when developing a protocol for testing the hemolytic activity of NPs, it is necessary to consider all four factors.

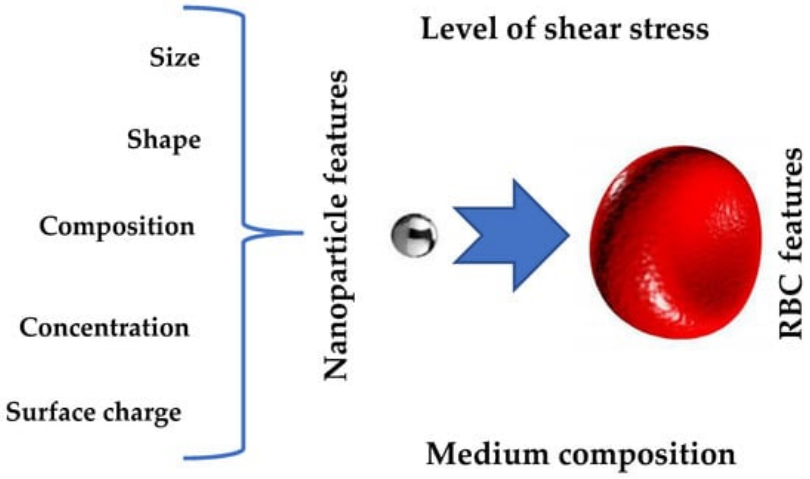

Figure 2. Factors that affect the hemolytic activity of nanoparticles.

References

1. Ding, H.; Zhang, J.; Zhang, F.; Xu, Y.; Liang, W.; Yu, Y. Nanotechnological approaches for diagnosis and treatment of o varian cancer: A review of recent trends. Drug Deliv. 2022, 29, 3218–3232.
2. Parhiz, H.; Khoshnejad, M.; Myerson, J.W.; Hood, E.; Patel, P.N.; Brenner, J.S.; Muzykantov, V.R. Unintended effects of drug carriers: Big issues of small particles. Adv. Drug Deliv. Rev. 2018, 130, 90–112.
3. Ding, Y.N.; Xue, M.; Tang, Q.S.; Wang, L.J.; Ding, H.Y.; Li, H.; Gao, C.C.; Yu, W.P. Immunotherapy-based novel nanopa rticles in the treatment of gastrointestinal cancer: Trends and challenges. World J. Gastroenterol. 2022, 28, 5403–5419.
4. Buzea, C.; Pacheco, I.I.; Robbie, K. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases 2007, 2, M R17–MR71.
5. Malatesta, M. Transmission Electron Microscopy as a Powerful Tool to Investigate the Interaction of Nanoparticles with Subcellular Structures. Int. J. Mol. Sci. 2021, 22, 12789.
6. Yohan, D.; Chithrani, B.D. Applications of nanoparticles in nanomedicine. J. Biomed. Nanotechnol. 2014, 10, 2371–239 2.
7. Chen, F.; Hong, H.; Shi, S.; Goel, S.; Valdovinos, H.F.; Hernandez, R.; Theuer, C.P.; Barnhart, T.E.; Cai, W. Engineering of Hollow Mesoporous Silica Nanoparticles for Remarkably Enhanced Tumor Active Targeting Efficacy. Sci. Rep. 2014, 4, 5080.
8. Gohari, G.; Mohammadi, A.; Akbari, A.; Panahirad, S.; Dadpour, M.R.; Fotopoulos, V.; Kimura, S. Titanium dioxide nano particles (TiO2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attrib utes of Dracocephalum moldavica. Sci. Rep. 2020, 10, 912.
9. Jiang, Y.; Zheng, W.; Tran, K.; Kamilar, E.; Bariwal, J.; Ma, H.; Liang, H. Hydrophilic nanoparticles that kill bacteria whil e sparing mammalian cells reveal the antibiotic role of nanostructures. Nat. Commun. 2022, 13, 197.
10. Loiseau, A.; Asila, V.; Boitel-Aullen, G.; Lam, M.; Salmain, M.; Boujday, S. Silver-Based Plasmonic Nanoparticles for an d Their Use in Biosensing. Biosensors 2019, 9, 78.
11. Meen, T.-H.; Tsai, J.-K.; Chao, S.-M.; Lin, Y.-C.; Wu, T.-C.; Chang, T.-Y.; Ji, L.-W.; Water, W.; Chen, W.-R.; Tang, I.-T.; et al. Surface plasma resonant effect of gold nanoparticles on the photoelectrodes of dye-sensitized solar cells. Nanoscal e Res. Lett. 2013, 8, 450.
12. Petithory, T.; Pieuchot, L.; Josien, L.; Ponche, A.; Anselme, K.; Vonna, L. Size-Dependent Internalization Efficiency of M acrophages from Adsorbed Nanoparticle-Based Monolayers. Nanomaterials 2021, 11, 1963.
13. Kher, C.; Kumar, S. The Application of Nanotechnology and Nanomaterials in Cancer Diagnosis and Treatment: A Revi ew. Cureus 2022, 14, e29059.
14. Moradpoor, H.; Safaei, M.; Mozaffari, H.R.; Sharifi, R.; Imani, M.M.; Golshah, A.; Bashardoust, N. An overview of recent progress in dental applications of zinc oxide nanoparticles. RSC Adv. 2021, 11, 21189–21206.

15. Murthy, S.K. Nanoparticles in modern medicine: State of the art and future challenges. Int. J. Nanomed. 2007, 2, 129–141.

16. Nel, A.; Xia, T.; Madler, L.; Li, N. Toxic Potential of Materials at the Nanolevel. Science 2006, 311, 622–627.

17. Xia, T.; Kovochich, M.; Liong, M.; Mädler, L.; Gilbert, B.; Shi, H.; Yeh, J.I.; Zink, J.I.; Nel, A.E. Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. ACS Nano 2008, 2, 2121–2134.

18. Donahue, N.D.; Acar, H.; Wilhelm, S. Concepts of nanoparticle cellular uptake, intracellular trafficking, and kinetics in nanomedicine. Adv. Drug Deliv. Rev. 2019, 143, 68–96.

19. Foroozandeh, P.; Aziz, A.A. Insight into Cellular Uptake and Intracellular Trafficking of Nanoparticles. Nanoscale Res. Lett. 2018, 13, 339.

20. De La Cruz, G.G.; Rodríguez-Fragoso, P.; Reyes-Esparza, J.; Rodríguez-López, A.; Gómez-Cansino, R.; Rodriguez-Fragoso, L. Interaction of Nanoparticles with Blood Components and Associated Pathophysiological Effects. In Unraveling the Safety Profile of Nanoscale Particles and Materials; de Casto Gomez, A.F.S.M., Ed.; IntechOpen: London, UK, 2018; pp. 168–180.

21. Fard, J.K.; Jafari, S.; Eghbal, M.A. A Review of Molecular Mechanisms Involved in Toxicity of Nanoparticles. Adv. Pharm. Bull. 2015, 5, 447–454.

22. Greish, K.; Thiagarajan, G.; Ghandehari, H. In Vivo Methods of Nanotoxicology. Methods Mol. Biol. 2012, 926, 235–253.

23. Santamaria, A. Historical Overview of Nanotechnology and Nanotoxicology. Methods Mol. Biol. 2012, 926, 1–12.

24. Luyts, K.; Napierska, D.; Nemery, B.; Hoet, P.H.M. How physico-chemical characteristics of nanoparticles cause their toxicity: Complex and unresolved interrelations. Environ. Sci. Process. Impacts 2013, 15, 23–38.

25. Wu, Y.-L.; Putcha, N.; Ng, K.W.; Leong, D.T.; Lim, C.T.; Loo, S.C.J.; Chen, X. Biophysical Responses upon the Interaction of Nanomaterials with Cellular Interfaces. Accounts Chem. Res. 2012, 46, 782–791.

26. Oberdörster, G.; Maynard, A.; Donaldson, K.; Castranova, V.; Fitzpatrick, J.; Ausman, K.; Carter, J.; Karn, B.; Kreyling, W.; Lai, D.; et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Part. Fibre Toxicol. 2005, 2, 8.

27. de la Harpe, K.M.; Kondiah, P.P.; Choonara, Y.E.; Marimuthu, T.; du Toit, L.C.; Pillay, V. The Hemocompatibility of Nanoparticles: A Review of Cell–Nanoparticle Interactions and Hemostasis. Cells 2019, 8, 1209.

28. Stone, V.; Johnston, H.; Schins, R.P.F. Development of in vitro systems for nanotoxicology: Methodological considerations. Crit. Rev. Toxicol. 2009, 39, 613–626.

29. Savage, D.T.; Hilt, J.Z.; Dziubla, T.D. In Vitro Methods for Assessing Nanoparticle Toxicity. Methods Mol. Biol. 2019, 1894, 1–29.

30. Odeyemi, S.W.; De La Mare, J.; Edkins, A.L.; Afolayan, A.J. In vitro and in vivo toxicity assessment of biologically synthesized silver nanoparticles from Elaeodendron croceum. J. Complement. Integr. Med. 2019, 16.

31. Pardeshi, S.R.; More, M.P.; Patil, P.B.; Mujumdar, A.; Naik, J.B. Statistical optimization of voriconazole nanoparticles loaded carboxymethyl chitosan-poloxamer based in situ gel for ocular delivery: In vitro, ex vivo, and toxicity assessment. Drug Deliv. Transl. Res. 2022, 12, 3063–3082.

32. Yazhiniprabha, M.; Vaseeharan, B. In vitro and in vivo toxicity assessment of selenium nanoparticles with significant larvicidal and bacteriostatic properties. Mater. Sci. Eng. C Mater. Biol. Appl. 2019, 103, 109763.

33. Yazhiniprabha, M.; Vaseeharan, B.; Sonawane, A.; Behera, A. In vitro and In vivo toxicity assessment of phytofabricated ZnO nanoparticles showing bacteriostatic effect and larvicidal efficacy against Culex quinquefasciatus. J. Photochem. Photobiol. B Biol. 2019, 192, 158–169.

34. Basith, S.; Manavalan, B.; Shin, T.H.; Park, C.B.; Lee, W.-S.; Kim, J.; Lee, G. The Impact of Fine Particulate Matter 2.5 on the Cardiovascular System: A Review of the Invisible Killer. Nanomaterials 2022, 12, 2656.

35. Nemmar, A.; Hoet, P.; Vanquickenborne, B.; Dinsdale, D.; Thomeer, M.; Hoylaerts, M.; Vanbilloen, H.; Mortelmans, L.; Nemery, B. Passage of Inhaled Particles Into the Blood Circulation in Humans. Circulation 2002, 105, 411–414.

36. Nemmar, A.; Vanbilloen, H.; Hoylaerts, M.F.; Hoet, P.H.M.; Verbruggen, A.; Nemery, B. Passage of Intratracheally Instilled Ultrafine Particles from the Lung into the Systemic Circulation in Hamster. Am. J. Respir. Crit. Care Med. 2001, 164, 1665–1668.

37. Blank, F.; von Garnier, C.; Gehr, P.; Rothen-Rutishauser, B. Translocation across the Air–Blood Tissue Barrier. In Nanoparticles in the Lung, 1st ed.; Tsuda, A., Gehr, P., Eds.; CRC Press: Boca Raton, FL, USA, 2015.

38. Barshtein, G.; Livshits, L.; Shvartsman, L.D.; Shlomai, N.O.; Yedgar, S.; Arbell, D. Polystyrene Nanoparticles Activate E rythrocyte Aggregation and Adhesion to Endothelial Cells. Cell Biophys. 2015, 74, 19–27.

39. Han, Y.; Wang, X.; Dai, H.; Li, S. Nanosize and Surface Charge Effects of Hydroxyapatite Nanoparticles on Red Blood Cell Suspensions. ACS Appl. Mater. Interfaces 2012, 4, 4616–4622.

40. Guo, S.; Shi, Y.; Liang, Y.; Liu, L.; Sun, K.; Li, Y. Relationship and improvement strategies between drug nanocarrier ch aracteristics and hemocompatibility: What can we learn from the literature. Asian J. Pharm. Sci. 2021, 16, 551–576.

41. Sarma, D.K.; Dubey, R.; Samarth, R.M.; Shubham, S.; Chowdhury, P.; Kumawat, M.; Verma, V.; Tiwari, R.R.; Kumar, M. The Biological Effects of Polystyrene Nanoplastics on Human Peripheral Blood Lymphocytes. Nanomaterials 2022, 12, 1632.

42. Shimizu, Y.; Isoda, K.; Tezuka, E.; Yufu, T.; Nagai, Y.; Ishida, I.; Tezuka, M. Influence of 50-nm polystyrene particles in i nducing cytotoxicity in mice co-injected with carbon tetrachloride, cisplatin, or paraquat. Die Pharm. 2012, 67, 712–714.

43. Canesi, L.; Ciacci, C.; Bergami, E.; Monopoli, M.; Dawson, K.; Papa, S.; Canonico, B.; Corsi, I. Evidence for immunom odulation and apoptotic processes induced by cationic polystyrene nanoparticles in the hemocytes of the marine bivalv e Mytilus. Mar. Environ. Res. 2015, 111, 34–40.

44. Loos, C.; Syrovets, T.; Musyanovych, A.; Mailänder, V.; Landfester, K.; Nienhaus, G.U.; Simmet, T. Functionalized polys tyrene nanoparticles as a platform for studying bio–nano interactions. Beilstein J. Nanotechnol. 2014, 5, 2403–2412.

45. Nurunnabi, M.; Khatun, Z.; Huh, K.M.; Park, S.Y.; Lee, D.Y.; Cho, K.J.; Lee, Y.-K. In Vivo Biodistribution and Toxicology of Carboxylated Graphene Quantum Dots. ACS Nano 2013, 7, 6858–6867.

46. Poland, C.A.; Duffin, R.; Kinloch, I.; Maynard, A.; Wallace, W.A.H.; Seaton, A.; Stone, V.; Brown, S.; MacNee, W.; Donal dson, K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot stu dy. Nat. Nanotechnol. 2008, 3, 423–428.

47. Chen, G.; Xiong, S.; Jing, Q.; van Gestel, C.A.; van Straalen, N.M.; Roelofs, D.; Sun, L.; Qiu, H. Maternal exposure to p olystyrene nanoparticles retarded fetal growth and triggered metabolic disorders of placenta and fetus in mice. Sci. Tota l Environ. 2022, 854, 158666.

48. Fan, X.; Wei, X.; Hu, H.; Zhang, B.; Yang, D.; Du, H.; Zhu, R.; Sun, X.; Oh, Y.; Gu, N. Effects of oral administration of po lystyrene nanoplastics on plasma glucose metabolism in mice. Chemosphere 2021, 288, 132607.

49. Li, D.; Sun, W.; Jiang, X.; Yu, Z.; Xia, Y.; Cheng, S.; Mao, L.; Luo, S.; Tang, S.; Xu, S.; et al. Polystyrene nanoparticles e nhance the adverse effects of di-(2-ethylhexyl) phthalate on male reproductive system in mice. Ecotoxicol. Environ. Sa f. 2022, 245, 114104.

50. Yasin, N.A.; El-Naggar, M.E.; Ahmed, Z.S.O.; Galal, M.K.; Rashad, M.M.; Youssef, A.M.; Elleithy, E.M. Exposure to Poly styrene nanoparticles induces liver damage in rat via induction of oxidative stress and hepatocyte apoptosis. Environ. T oxicol. Pharmacol. 2022, 94, 103911.

51. Sukhanova, A.; Bozrova, S.; Sokolov, P.; Berestovoy, M.; Karaulov, A.; Nabiev, I. Dependence of Nanoparticle Toxicity o n Their Physical and Chemical Properties. Nanoscale Res. Lett. 2018, 13, 44.

52. Cheng, L.-C.; Jiang, X.; Wang, J.; Chen, C.; Liu, R.-S. Nano–bio effects: Interaction of nanomaterials with cells. Nanosc ale 2013, 5, 3547–3569.

53. Yu, T.; Malugin, A.; Ghandehari, H. Impact of Silica Nanoparticle Design on Cellular Toxicity and Hemolytic Activity. ACS Nano 2011, 5, 5717–5728.

54. Mohanty, J.G.; Nagababu, E.; Rifkind, J.M. Red blood cell oxidative stress impairs oxygen delivery and induces red blo od cell aging. Front. Physiol. 2014, 5, 84.

55. Yedgar, S.; Hovav, T.; Barshtein, G. Red blood cell intercellular interactions in oxidative stress states. Clin. Hemorheol. Microcirc. 1999, 21, 189–193.

56. Ben-Hur, E.; Barshtein, G.; Chen, S.; Yedgar, S. Photodynamic Treatment of Red Blood Cell Concentrates For Virus In activation Enhances Red Blood Cell Aggregation: Protection with Antioxidants. Photochem. Photobiol. 1997, 66, 509–5 12.

57. Barshtein, G.; Arbell, D.; Yedgar, S. Hemolytic Effect of Polymeric Nanoparticles: Role of Albumin. IEEE Trans. NanoBi osci. 2011, 10, 259–261.

58. Ferenc, M.; Katir, N.; Miłowska, K.; Bousmina, M.; Majoral, J.-P.; Bryszewska, M.; El Kadib, A. Haemolytic activity and c ellular toxicity of SBA-15-type silicas: Elucidating the role of the mesostructure, surface functionality and linker length. J. Mater. Chem. B 2015, 3, 2714–2724.

59. ASTM E2524-08; Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles. ASTM: West Conshohoc ken, PE, USA, 2008.

60. Dobrovolskaia, M.A.; McNeil, S.E. Understanding the correlation between in vitro and in vivo immunotoxicity tests for n anomedicines. J. Control. Release 2013, 172, 456–466.
61. Luna, L.A.V.; Martinez, D.S.T.; Alves, O.L. Nanomaterials: From Current Methods to Biomolecular Surface Chemistry In teractions. In Nanotoxicology: Materials, Methodologies and Assessments; Durán, N., Guterres, S.S., Alves, O.L., Eds.; Springer: Sao Paulo, Brazil, 2014.
62. Martinez, D.S.T.; Paula, A.J.; Fonseca, L.C.; Luna, L.A.V.; Silveira, C.P.; Durán, N.; Alves, O.L. Monitoring the Hemolyti c Effect of Mesoporous Silica Nanoparticles after Human Blood Protein Corona Formation. Eur. J. Inorg. Chem. 2015, 2 015, 4595–4602.
63. Choi, J.; Reipa, V.; Hitchins, V.M.; Goering, P.L.; Malinauskas, R.A. Physicochemical Characterization and In Vitro Hem olysis Evaluation of Silver Nanoparticles. Toxicol. Sci. 2011, 123, 133–143.
64. A Love, S.; Thompson, J.W.; Haynes, C.L. Development of screening assays for nanoparticle toxicity assessment in hu man blood: Preliminary studies with charged Au nanoparticles. Nanomedicine 2012, 7, 1355–1364.
65. Zhao, Y.; Sun, X.; Zhang, G.; Trewyn, B.G.; Slowing, I.I.; Lin, V.S.-Y. Interaction of Mesoporous Silica Nanoparticles wit h Human Red Blood Cell Membranes: Size and Surface Effects. ACS Nano 2011, 5, 1366–1375.
66. Li, Y.; Liu, J.; Zhong, Y.; Zhang, D.; Wang, Z.; An, Y.-L.; Lin, M.; Gao, Z.; Zhang, J. Biocompatibility of composite magne tic nanoparticles in vitro and in vivo. Int. J. Nanomed. 2011, 6, 2805–2819.
67. Lu, S.; Duffin, R.; Poland, C.; Daly, P.; Murphy, F.; Drost, E.; MacNee, W.; Stone, V.; Donaldson, K. Efficacy of Simple S hort-Term in Vitro Assays for Predicting the Potential of Metal Oxide Nanoparticles to Cause Pulmonary Inflammation. Environ. Health Perspect. 2009, 117, 241–247.
68. Cho, W.-S.; Duffin, R.; Bradley, M.; Megson, I.L.; MacNee, W.; Lee, J.K.; Jeong, J.; Donaldson, K. Predictive value of in vitro assays depends on the mechanism of toxicity of metal oxide nanoparticles. Part. Fibre Toxicol. 2013, 10, 55.
69. Arts, J.H.E.; Hadi, M.; Irfan, M.-A.; Keene, A.M.; Kreiling, R.; Lyon, D.; Maier, M.; Michel, K.; Petry, T.; Sauer, U.G.; et a l. A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping). Regul. Toxicol. Phar macol. 2015, 71, S1–S27.
70. Huisjes, R.; Bogdanova, A.; van Solinge, W.W.; Schiffelers, R.M.; Kaestner, L.; van Wijk, R. Squeezing for Life–Properti es of Red Blood Cell Deformability. Front. Physiol. 2018, 9, 656.
71. Pan, D.; Vargas-Morales, O.; Zern, B.; Anselmo, A.C.; Gupta, V.; Zakrewsky, M.; Mitragotri, S.; Muzykantov, V. The Effe ct of Polymeric Nanoparticles on Biocompatibility of Carrier Red Blood Cells. PLoS ONE 2016, 11, e0152074.
72. Kim, M.J.; Shin, S. Toxic effects of silver nanoparticles and nanowires on erythrocyte rheology. Food Chem. Toxicol. 20 14, 67, 80–86.
73. Lin, Y.-S.; Haynes, C.L. Impacts of Mesoporous Silica Nanoparticle Size, Pore Ordering, and Pore Integrity on Hemolyti c Activity. J. Am. Chem. Soc. 2010, 132, 4834–4842.