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Injectable Nanosystems and Inherent Nanoparticulate-Serum Interactions

Mershen Govender, Sunaina Indermun, Pradeep Kumar, Yahya E. Choonara, and Viness Pillay

Abstract

The development of nanoparticulate systems has been shown to be highly effective in the advancement of pharmaceutical drug delivery technology. The use of these systems, however, has been shown to have significant physical and chemical effects on blood constituents, especially when delivered via intravenous injection. These effects have been shown to impact not only the individual blood constituents and their physiological roles but also the efficacy of the nanoparticulate formulation as well. Numerous studies have therefore focused on the impacts of nanoparticulate delivery on blood and have detailed the modifications undertaken to ensure hemocompatibility. This chapter will therefore focus on the interactions between nanoparticulate delivery systems and blood constituents upon delivery, the effect of these interactions and the newer research that has been performed to overcome the known issues of nanoparticle blood compatibility.

M. Govender · S. Indermun · P. Kumar
Y. E. Choonara · V. Pillay (✉)
Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Gauteng, South Africa
e-mail: viness.pillay@wits.ac.za

Keywords

Nanoparticulate system · Injectable delivery · Serum-Protein interactions · Hemocompatibility · Blood coagulation

1 Introduction

Nanosystems have been widely developed over the past few decades for their enhanced drug delivery applications. These systems, while effective, however, do exert an inherent effect on the physiological environment upon administration. Injectable systems which deliver drug directly to system circulation instantaneously come into physical contact with blood constituents. These include blood cells, platelets, and proteins, each of which have a different interaction upon contact with nanosystems (Fig. 26.1) (De La Cruz et al. 2017).

The interaction that occurs upon administration of nanosystems is dependent largely on the properties of the drug delivery system with the size, surface charge, and properties and morphology having a significant impact on the viability and functionality of the respective blood constituents. With these interactions, research has focused on the hemocompatibility of drugs as a significant component of nanoparticle development. This has resulted in significant modifica-

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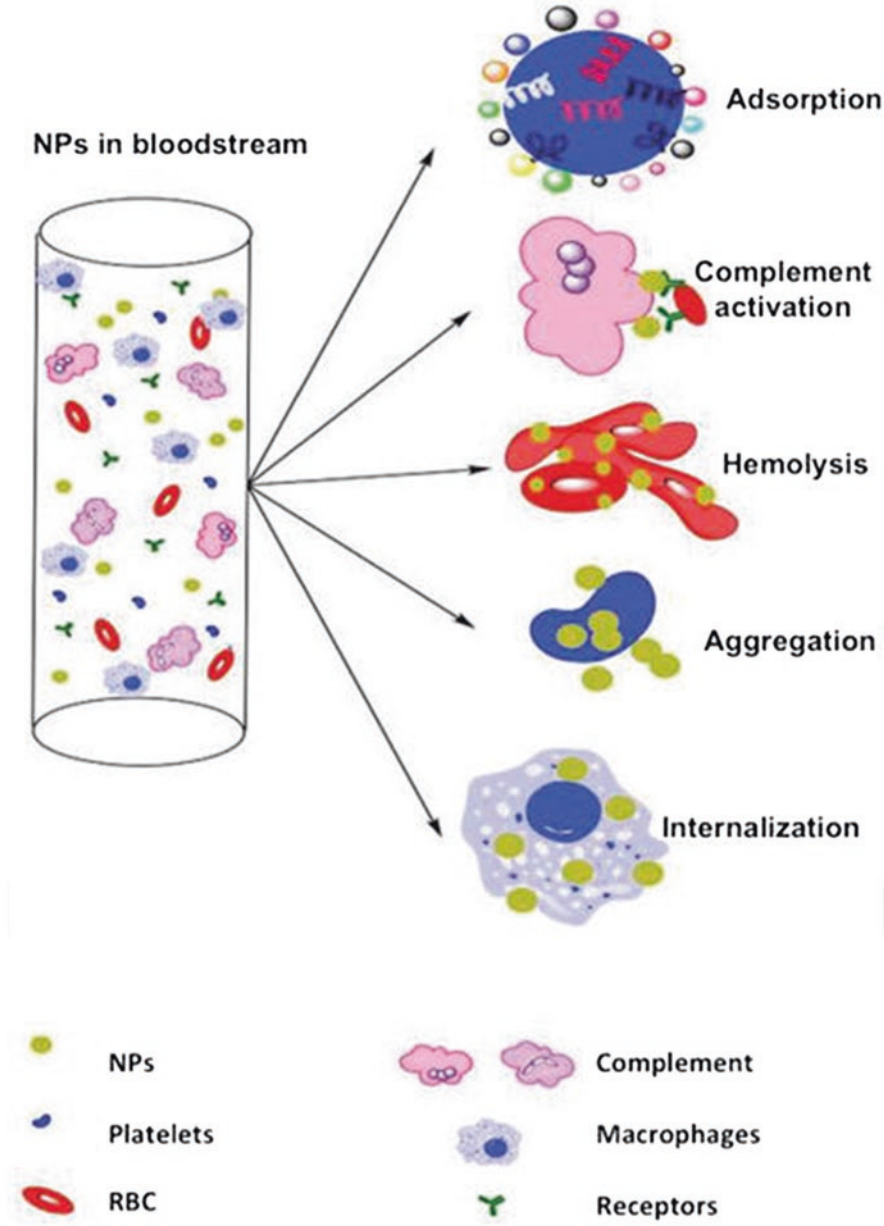

Fig. 26.1 Interaction of nanoparticles with bloodstream components and physiological effects. (Reproduced from De La Cruz et al. (2017) © 2017 The Author(s) Licensee IntechOpen)

tions to the developed particles to ensure that limited side effects occur upon administration.

With the need for the development of hemocompatible nanoparticles devoid of significant nanoparticle-serum constituent interactions, research has further used these interactions for the advancement of patient treatment through the development of systems such as platelet aggregates or immune enhancers (Gu 2018). These systems using innovative technologies have been shown to successfully utilize physiological processes to enhance nanoparticle efficacy.

This chapter will therefore focus on the impact of nanoparticulate delivery, primarily through the

intravenous route, on serum constituent efficacy, viability, and functionality. These constituents which include serum proteins, platelets, red blood cells, and immune cells interact with nanoparticulate in various ways, each with a potential physiological impact (De La Cruz et al. 2017; Zolnik et al. 2010; Gamucci et al. 2014). Research that has utilized these interactions for the advancement of therapeutic treatment has also been highlighted and discussed.

2 Interaction Between Nanoparticulate Systems and Serum Proteins

Numerous articles have been published highlighting the hemocompatibility of developed injectable nanoparticulate drug delivery systems. Research has shown that the physical interaction between nanoparticles and serum proteins often results in the development of the protein corona, which occurs when ionic-charged nanoparticles interact with the colloidal surface of serum proteins forming a biomolecular adsorption layer (De La Cruz et al. 2017; Abstiens et al. 2019). This protein corona consists of both soft and hard layers formed around the nanoparticle surface instantly upon administration of the nanoparticles due to interactions between the serum proteins and nanoparticles and is a result of London dispersion, Coulomb forces, p–p stacking, hydrogen-bonds and hydrophobic interactions. Particle properties such as particle size, surface curvature, charge, hydrophobicity, and topography have also been recognized to determine the kinetic degree and identity of the protein corona formation (Abstiens et al. 2019). The effect of the protein corona formation is significant as conformational changes of the protein adsorbed onto the surface of nanoparticles may result in new epitope exposure as well as functional and affinity changes (Parveen et al. 2017; Zhang et al. 2019; Liu et al. 2020). Due to these changes, variations of biological function may occur and as a result may cause potential biological injury. In order to assess the conformational changes, various spectroscopy techniques are generally employed as highlighted in Fig. 26.2.

A study undertaken by Abstiens et al. (Abstiens et al. 2019) investigated the interaction of functionalized polymeric nanoparticles with varying hydrophobicity and surface charge on serum proteins with emphasis on cargo leaching and colloidal stability from the nanoparticles. The results of this study detailed that the developed zwitterionic nanoparticles were least affected by the protein corona development as compared to the polymeric colloids functionalized with uncharged methoxy groups or negatively charged carboxylate groups. The reason given for this result was that the zwitterionic nanoparticles provide only a limited probability for serum proteins to interact via hydrophobic or electrostatic forces. The study also determined that positively charged nanoparticles have a greater interaction with serum proteins when compared to the other nanoparticles evaluated. This resulted in a decreased colloidal stability and increased leaching of drug from the nanoparticle core.

The protein corona furthermore has significant impacts on the cellular environment within the body. The degree of these effects however varies greatly from insignificant to apoptosis of the respective cell. The comprehensive review of the impact of the formed protein corona and its impact of nanoparticles and cells can be found in Table 26.1.

3 Interaction Between Nanosystems and Serum Blood Cells

Interactions between delivered nanosystems and serum blood cells are important to determine that unwanted side effects associated with blood cell changes do not occur. Various studies have been undertaken to investigate the effects of prepared nano-formulations on serum blood cells. One such study was undertaken by Bruckman et al. (Bruckman et al. 2014) and detailed the hemocompatibility of virus nanorods and nanospheres in mice serum specifically with hemolysis and blood coagulation (Fig. 26.3). The PEGylated tobacco mosaic nanosystems administered intra-

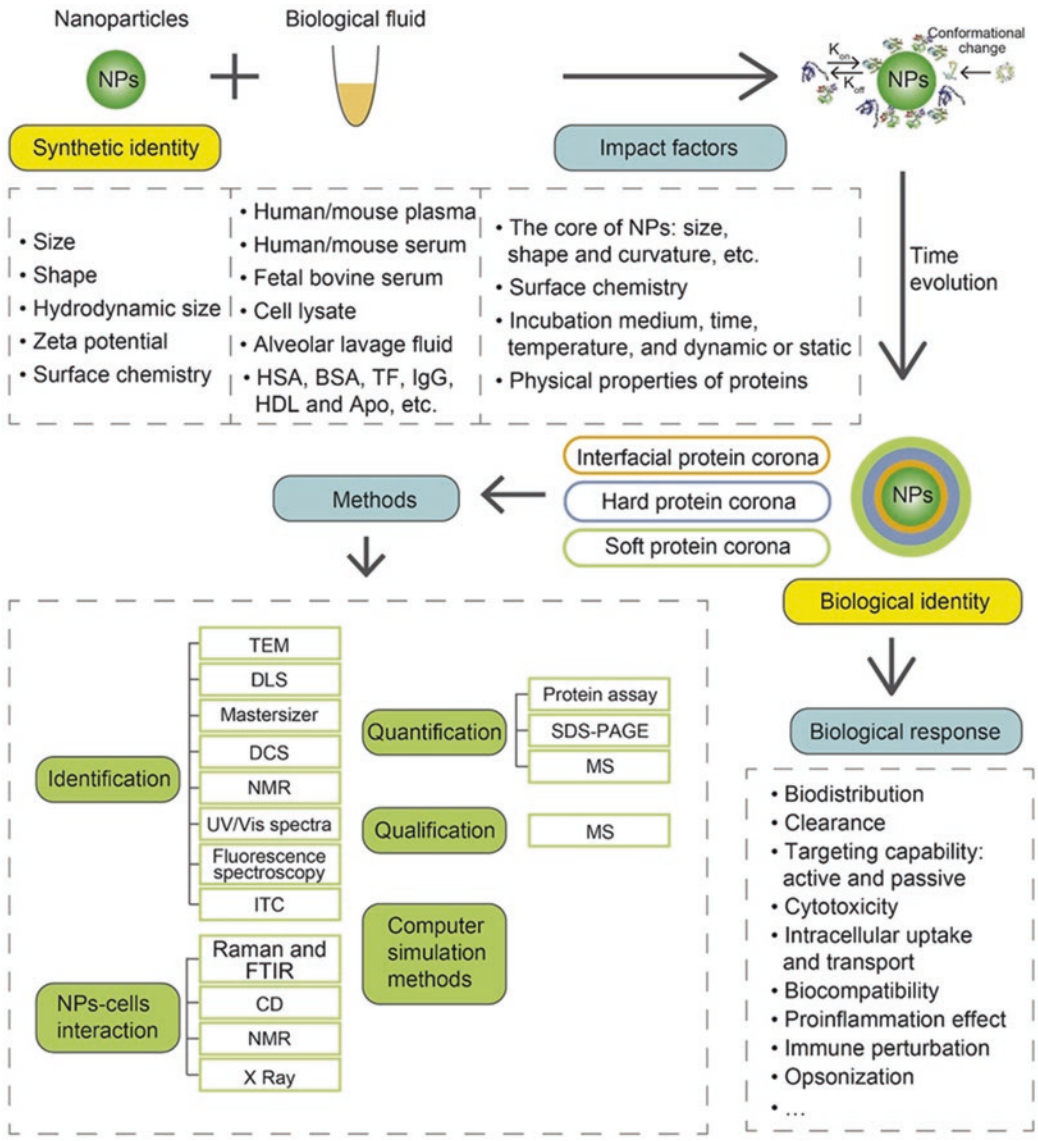

Fig. 26.2 The experimental workflow of protein corona and impact factors. *TEM* Transmission electron microscope, *DLS* Dynamic light scattering, *DCS* Differential centrifugation sedimentation, *NMR* Nuclear magnetic resonance, *ITC* Isothermal titration calorimetry, *FTIR* Fourier transform infrared, *CD* circular dichroism, *MS* Mass spectrometry. (Reproduced with permission from Liu et al. (2020) © 2019 Elsevier Ltd)

venously were determined to have minimal effects on red blood cell integrity and blood coagulation. While the properties of the developed systems in this study were shown to have positive results with regard to hemocompatibility, this study highlights the importance of evaluating the effects of nanosystems on blood cell properties and integrity.

Another study undertaken by Li and coworkers (LiH-C et al. 2013) investigated the hemocompatibility effects of monocrystalline nanodiamonds (NDs). The hemolytic ability was assessed in human red blood cells (RBCs). It was found that there was no RBC destruction irrespective of particle size (35–500 nm) and that the particles lacked thrombogenic activity.

Table 26.1 The impact of protein corona on the interaction between nanoparticles and cells

Type of protein corona	Nanoparticles	Size by TEM (nm)	Cell type	Level of cellular uptake	Level of cytotoxicity/type of immunotoxicity
DMEM with 10% FBS	Ag-CIT NPs	15 ± 3	NIH-3 T3	Up	Apoptosis
HSA	Ag-PVP NPs Ag-CIT NPs	20	SH-SY5Y HepG2	Down Down	Up Up
HSA	Ag-CIT NPs	20 110	HEK	Down Down	–
	Ag-SiO_2 NPs	20 120		ns ns	
IgG	Ag-CIT NPs	20 110	Down Down		
	Ag-SiO_2 NPs	20 120	Up Ns		
Tf	Ag-CIT NPs	20 110	Down Down		
	Ag-SiO_2 NPs	20 120	ns ns		
BSA	Ag-CIT NPs	20	RAECs	Down	ER stress
HDL				Down	ER stress
FBS				Down	ER stress
HSA BSA HDL	Ag-CIT NPs	20	RLECs and RAECs	Down	Cell activation; inducing IL-6 expression
10% FBS	Ag-CIT NPs 20 Ag-PVP NPs	20 110 20 110	RAW264.7	Up Up Up ns	Down Down Down Down
BSA	Ag-CIT NPs	20 110		Up Up	Down ns
	Ag-PVP NPs	20 110		Down ns	Down ns
Fibrinogen	Ag-PAA NPs	5	THP-1	–	Pro-inflammatory effect
Clusterin	Ag NPs	10	dTHP-1	Down	–
	SiO_2 NPs	70			
10% HS	SiO_2 NPs	50	M1 macrophages M2 macrophages	Down Down	–
10% heat-inactivated HS			M1 macrophages M2 macrophages	Block Block	
HAS			M1 macrophages M2 macrophages	Block Block	
10% FBS	SiO2 NPs	12 and 50	A549 and RAW264.7	Up	Down
Murine lung tissue fluid (TGF-b1)	SiO2 NPs	100	A549 cells	–	Epithelial-mesenchymal transition (EMT)
55% HP	BPEI-AuNPs LA-AuNPs PEG-AuNPs	40	HUVEC	Down Down no	Down Down Down

(continued)

Table 26.1 (continued)

Type of protein corona	Nanoparticles	Size by TEM (nm)	Cell type	Level of cellular uptake	Level of cytotoxicity/type of immunotoxicity
HAS	BPEI-AuNPs LA-AuNPs PEG-AuNPs	40 and 80		Up Down Uo	Down Down Down
DMEM with 10% FBS	PEG-Au NPs	5, 20, and 50	RAW 264.7 HepG2	Down	–
HS	PS-COOH NPs PS-NH_2 NPs	100	3D HepG2 cells	Up	–
FBS	PS-COOH NPs	40	A549	Down	–
HS	PS-COOH NPs	50	hMSCs	Ns	–
Human ApoA-4				Down	
Human ApoC-3				Down	
Human AntIII				Ns	
THRB				Ns	
VTN				Ns	
ApoH				Up	
SP-A	CH-mNPs	110–180	Murine AMs	Up	–
BSA	PMO-mNPs PL-mNPs			Down	
FCS	PEI-SPIO NPs	182 by DLS	hBMECs	Down	
BSA	Fe3O4 NPs	20	HeLa cells	Down	Down
BTf				Down	Down
Big				Down	Down
BFG				Down	Down
FBS	MHA NPs	14 × 2	MC3T3-E1 cells	–	Cell proliferation
Tf	FePt-COOH	5.6	HeLa cells	Down	–
HAS	NPs			Down	
BSA	GO NPs	250.63 ± 9.76	A549 HEK 293	Down Up	–
FBS	CdS NPs	30	NR8383 cells	Down	Apoptosis
HP	BP QDs	5	dTHP-1	Up	Pro-inflammatory effect Immune perturbation
FBS	PM2.5	–	HLFs	–	Aberrant proliferation

FBS, fetal bovine serum; HS, human serum; HSA, human serum albumin; Apo, apolipoprotein; Ant, antithrombin; THRB, coagulation factors prothrombin; VTN, vitronectin; SP-A, surfactant protein-A; BSA, bovine serum albumin; FCS, fetal calf serum; Tf, transferrin; BFG, bovine fibrinogen; HDL, high-density lipoprotein; HP, human plasma; TGFb1, transforming growth factor-b1. CIT, citrate; PS, polystyrene; CH, chitosan; mNPs, magnetite NPs; PMO, poly-maleic-oleic acid; PL, phosphatidylcholine; PEI, polyethylenimine; SPIO, superparamagnetic iron oxide; BPEI, branched polyethylenimine; LA, lipoic acid; PEG, polyethyleneglycol; GO NPs, gelatin-oleic NPs; PV, polyvinylpyrrolidone; PAA, poly(acrylic acid); BP QDs, black phosphorus quantum dots; MHA, magnetic hydroxyapatite; NIH-3 T3 cells, mouse embryonic fibroblast; hMSCs, human mesenchymal stem cells; AMs, alveolar macrophages; hBMECs, human brain microvascular endothelial cells; HeLa cells, human cervix carcinoma cells; HepG2 cells, human hepatocellular liver carcinoma cell line; A549 cells, lung carcinoma cells; HPTCs, human proximal tubule cells; HUVECs, human umbilical vein endothelial cells; RAW 264.7, mouse leukemia cells of monocyte macrophage; HEK 293, human embryonic kidney cells; SH-SY5Y, human neuroblastoma cells; RAECs, rat aortic endothelial cells; RLECs, rat lung epithelial; dTHP-1, differentiated human leukemic monocyte; NR8383 cells, rat lung macrophage line; HLFs, human lung fibroblasts; MC3T3-E1 cells, mouse embryonic osteoblast precursor cells

Ns: no significant effect

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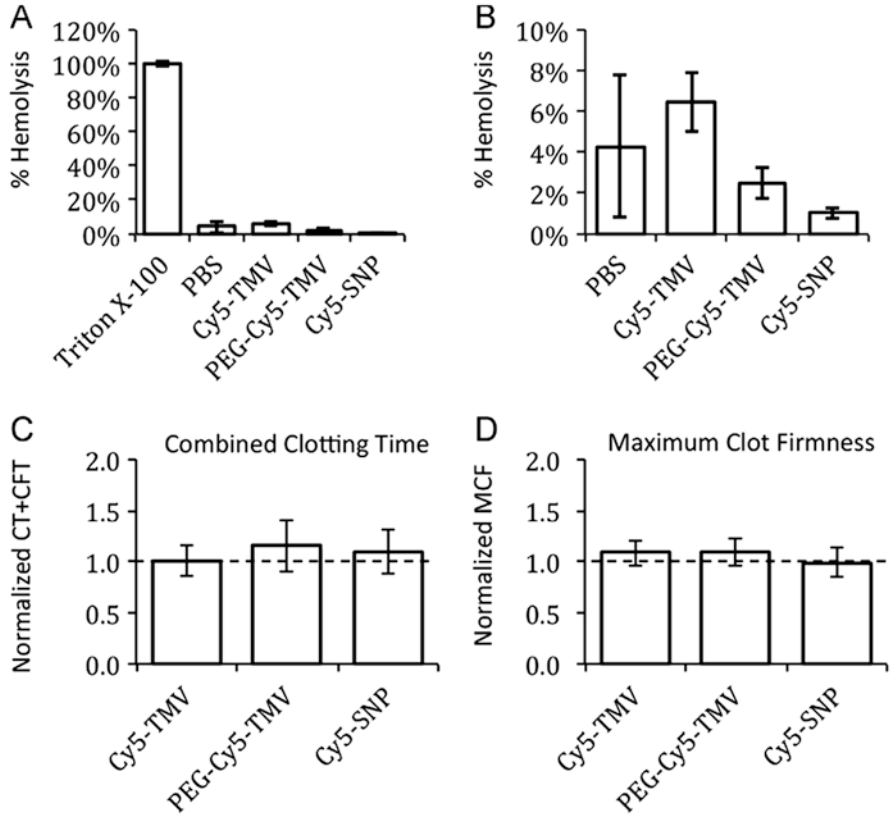

Fig. 26.3 Blood biocompatibility assays. (**a**) Red blood cell (RBC) hemolysis assay. (**b**) Zoomed in RBC hemolysis assay showing Cy5-TMV, PEG-Cy5-TMV, and Cy5-SNP do not lyse RBCs. Effect of Cy5-TMV, PEG-Cy5-TMV, and Cy5-SNP on (**c**) clotting (normalized to saline control) and (**d**) maximum clot firmness (MCF), measured in rotational thromboelastometry (ROTEM). There were no significant changes in the combined clotting time (CTþCFT) and maximum clot firmness compared to the saline control (dotted line). Error bars represent S.D. (Reproduced with permission from Bruckman et al. (2014) © 2013 Elsevier Inc)

Synthesized through high-pressure-high-temperature (HPHT) methods, the NDs were found to also be non-cytotoxic on human primary endothelial cells. The inflammatory cytokine levels (IL-1b and IL-6) were not found to be significantly elevated after intravenous injection into FVB mice.

Other studies that prepared hemocompatible nanosystems included the study undertaken by Palazzo and co-workers (Palazzo et al. 2019) who developed injectable liposome (Lipo-E4) and drug-in-cyclodextrin-in-liposome (DCL-E4) formulations for the delivery of estetrol (E4) for the treatment of neonatal hypoxic-ischemic encephalopathy (HIE). This estradiol metabolite was encapsulated in the formulation to enhance blood-brain barrier (BBB) permeation. In vitro tests were conducted on endothelial (EAhy926, HUVEC), neuronal, (Neuro2a) and BBB model cells (hCMEC/D3) and the hemocompatibility was assessed. The formulation displayed good hemocompatibility and no cellular toxicity. In addition, the protein corona formed was approximately 8 nm in size, and no hemolytic effect was observed highlighting a protein interaction but no significant effect on red blood cells. Azmi and co-workers (Azmi et al. 2016) also fabricated internally self-assembled "somes" or nanoparticles from a binary lipid system consisting of citrem and soy phosphatidylcholine. A range of lyotropic lamellar and non-lamellar liquid crystalline nanodispersions displayed increased hemocom-

patibility and a lack of late stage complement activation upon interaction with serum constituents.

Modification of the prepared nanosystems has also been determined to have a significant effect on hemocompatibility upon administration. Once such study by Datta and co-workers (Datta et al. 2017) was undertaken on the in vitro characteristics of titanium dioxide (TiO_2) nanoparticles. The nanoparticles were studied for their biocompatibility effects on human serum albumin, hepatocellular carcinoma (HepG2) cell line, and erythrocytes. The nanoparticles were functionalized with hydroxyl (hydroxylated (OH-TiO_2) titania), amine (aminosilane (NH2-TiO_2)), or thiol (mercaptosilane (SH-TiO_2)) moieties and the respective influence on nanoparticle toxicity and protein binding investigated. The hemolytic ability of the nanoparticles was found to be reduced by the functionalized nanoparticles, and the nanoparticles functionalized with amine moieties were found to significantly enhance hemocompatibility. Dose-dependent cytotoxicity was also observed from both the pristine and functionalized nanoparticles. Cell viability was further found to be increased when the nanoparticles were functionalized with the aminosilane or mercaptosilane moieties.

Lin and co-workers (Lin et al. 2019), by studying the effects of positively charged pH-responsive micelles on blood, proposed a biomimetic phosphorylcholine strategy to enhance the hemocompatibility of these types of nanoparticles (Fig. 26.4). The nanoparticles were designed to be able to form self-assembling biomimetic phosphorylcholine micelles from amphiphilic copolymers containing varying umbers of tertiary amino groups. The phosphorylcholine functioned to protect the positive charges induced by the amino groups and thereby improve hemocompatibility. No significant effect was demonstrated on the RBCs, platelet activation, and coagulation.

An additional study undertaken by Singh and co-workers (Singh et al. 2018) utilized 1,3β-glucan as an outer shell to paclitaxel-loaded chitosan nanoparticles (1,3β-Cs-PTX-NPs) for the chemotherapeutic treatment of malignant glioblastoma. The ligand-based targeting delivery system was evaluated in vitro in glioma stem cell line (C6) and a glioma cancer cell line (LN-18) where the nanoparticles displayed a higher cellular uptake and internalization in the C6 line as well as a higher in vitro efficacy against both cell lines. The study also evaluated the hemolytic potential of the 1,3β-Cs-PTX-NP formulation in RBCs from mice blood and was found to be significantly reduced as compared to paclitaxel thereby allowing for intravenous delivery. Moreover, extended release of paclitaxel was established, leading to the non-specific systemic toxicity of paclitaxel. Wang and co-workers (Wang et al. 2019) further synthesized a series of poly (N-isopropylacrylamide) (PNIPA)/layered double hydroxides (LDHs)/nano-hydroxyapatite (nano-HA) hydrogels. At 33 °C, the PNIPA/LDHs/HA composite hydrogels exhibited reversible sol-gel behavior. Hemolysis was assessed using human whole blood, and the gels were found to display a hemolysis percentage of ~1%. An additional study by Xu and co-workers (Xu et al. 2019) developed a multifunctional nucleic acid delivery nanosystem (TP-Gd/miRNA-ColIV) for thoracic aortic dissection (TAD) treatment. The nanosystem successfully delivered miR-145 to prevent TAD deterioration. Moreover, the system was found to display good blood compatibility and be nontoxic to organs.

4 Interaction Between Nanosystems and the Blood Coagulation System

The effect of nanoparticulate delivery on blood clotting has been highlighted previously to be significant in the prevention of unwanted side effects (Fröhlich 2016). These effects are as a result of the numerous cascading of signals that occur upon the physical adhering interaction between the platelets and the nanoparticle structure leading to fibrin cross-linking and clot formation. This can be of significance due to potential formation of potentially deadly thrombi which can lead to strokes or other cerebrovascular accidents (De La Cruz et al. 2017). The study of

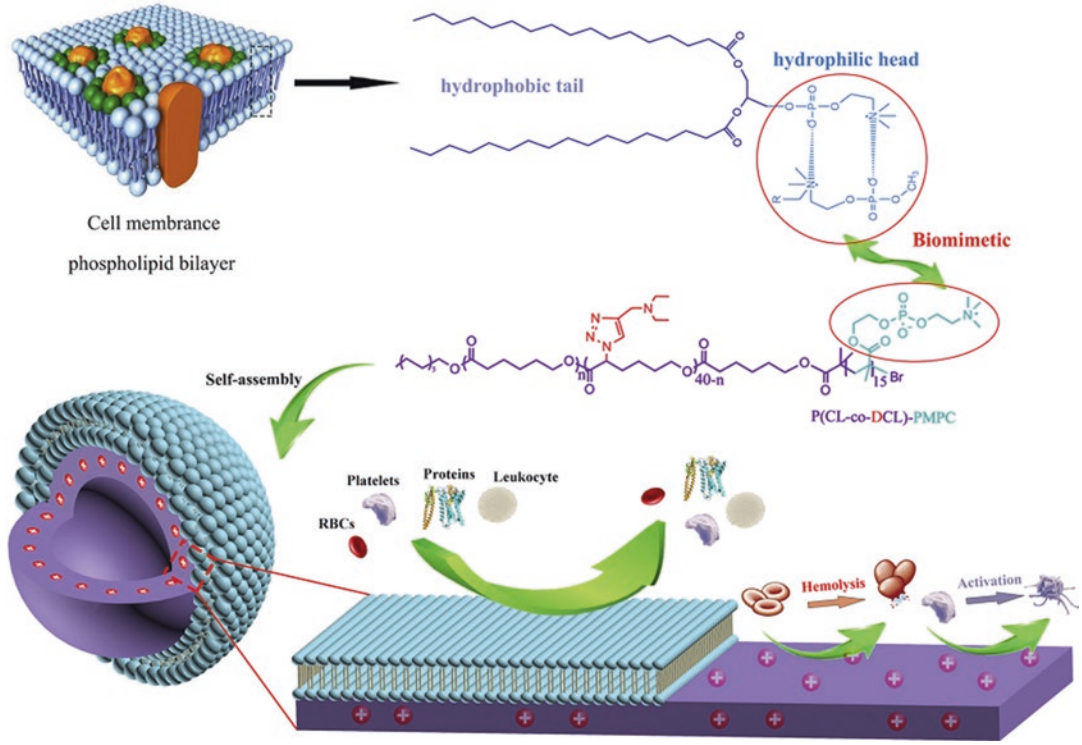

Fig. 26.4 Schematic illustration of the hemocompatibility of the micelles. (Reproduced with permission from Lin et al. (2019) © 2019 Elsevier B.V)

the effect of nanoparticle delivery on the coagulation system is therefore highly warranted and required.

The use of this interaction however can be advantageous as it allows for the controlling or modification of clotting functionality of the human body to exert required effects. In a study by Rajabi and co-workers (Rajabi et al. 2020), a nanocomposite hydrogel was fabricated for its potential as a sealant in surgical applications. This adhesive hydrogel contained thiolated gelatin (Gel-SH), gelatine methacrylate (GelMA), and polydopamine functionalized Laponite® (PD-LAP) which imparted an enhanced blood compatibility, blood clotting ability (2.25 min), and tissue adhesive strength to the nanocomposite gel (Fig. 26.5). In vitro cytocompatibility tests using L929 fibroblast cells revealed that after 5 days of various hydrogel culture, higher cell viability was demonstrated. The results also revealed a clotting time of more than 72% when in comparison to other tested sealants. The nanogel was found to be non-hemolytic with a hemolysis ratio of less than 5%.

Also, in the field of hydrogels, Zhao and coworkers (Zhao et al. 2018) developed injectable cryogels for hemorrhage and wound healing applications. The antibacterial conductive nanocomposite gels were composed of carbon nanotubes and glycidyl methacrylate functionalized quaternized chitosan. The hemostatic performance of the gels was assessed in mouse liver injury and mouse-tail amputation models by evaluating the bleeding and hemostatic times, whereby excellent hemostatic effects were observed. Additionally, the positive hemostatic effects were evaluated in the rabbit liver defect lethal non-compressible hemorrhage and standardized circular liver bleeding models. Results of these analyses revealed that the gels were found to also exhibit shape memory and wound healing properties. When evaluated against conventional hemostatic methods, the cryogels were also found to display superiority and a higher

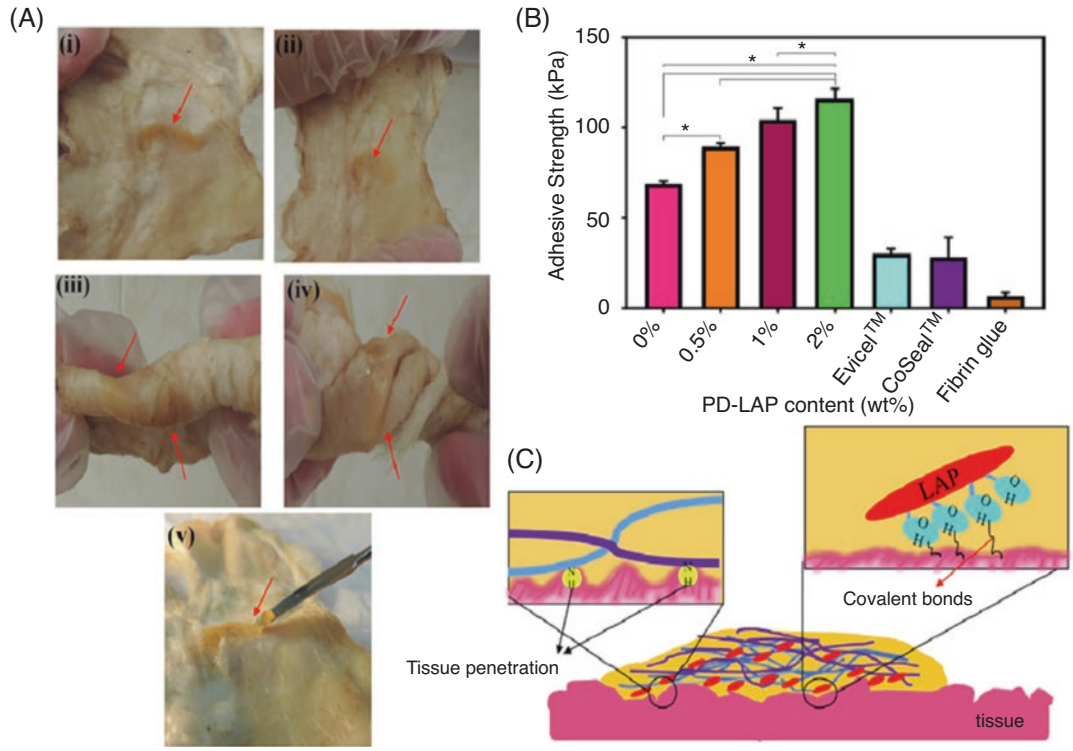

Fig. 26.5 Adhesive properties of nanocomposite hydrogels: (**a**) Photographs of gel-1% PD-LAP hydrogel adhered to sheepskin under (i) normal condition and under (ii) tensile, (iii) torsion, and (iv) twist deformation, confirming the high adhesion strength. (v) The nanocomposite hydrogels removed completely from the skin, demonstrating their highly cohesive strength. (**b**) Adhesive strength changes of nanocomposite hydrogels as a function of PD-LAP content. The adhesion strength of hydrogels was compared with the results of three commercial glues, with the data shown as means ± SD ($n = 3$) (*: $P < 0.05$). (**c**) Schematic representation of tissue penetration and new covalent bonds between tissue and nanocomposite hydrogels. (Reproduced with permission from Rajabi et al. (2020) ©2019 Elsevier Inc)

blood cell and platelet adhesion as well as activation and appreciable blood-clotting ability. Furthermore, the cryogels displayed excellent cytocompatibility and were found to be non-cytotoxic.

5 Nanoparticle Effects on Other Blood Constituents

The effects of nanoparticle interactions with serum constituents also extend beyond proteins, platelets, and red blood cells to include peripheral mononuclear cells such as lymphocytes, monocytes, natural killer cells, and granulocytes (De La Cruz et al. 2017). This is due to the administered nanoparticles often first being picked up by the phagocytic cells of the immune system which may lead to undesirable effects such as immunostimulation or immunosuppression. Further effects could also include inflammatory or autoimmune disorders or increases in the host's susceptibility to infections and cancer (Zolnik et al. 2010; Gamucci et al. 2014).

Additional interactions have also been noted previously to have a significant impact on the functionality of and communication between immune cells. This is due to the impact on exosomes which function in cell-to-cell communication (De La Cruz et al. 2017; Andersson-Willman et al. 2012). Research undertaken by Andersson-Willman et al.

(Andersson-Willman et al. 2012) noted the effects of TiO_2 and ZnO nanoparticles on dendritic cells, lymphocytes, and exosome production at sub-toxic concentrations. The results of this study detailed that the viability of the analyzed primary human peripheral blood mononuclear cells was not affected by the tested concentrations (1 to 100 μg/mL). However, a dose-dependent increase in cell death and caspase activity in monocyte-derived dendritic cells to ZnO was observed, which was not seen with the TiO_2 nanoparticles. The ZnO nanoparticles further induced a downregulation of FcγRIII (CD16) expression on NK cells noting that at the tested concentrations of ZnO nanoparticles, an effect on FcγR-mediated immune responses may occur.

These immune cells which can be induced through interaction with nanoparticles can further result in inflammation. Research has shown that the inflammatory response occurring as a result of administration of a drug delivery system is due to cytokine release. The properties of the number of different cytokines induced can be correlated with the surface charge of the particles delivered with highest number being for cationic micelles (13 cytokines) with zwitterionic (seven cytokines), neutral (three cytokines), and anionic micelles (one cytokine) significantly lower (Elsabahy and Wooley 2015). Cross-linking and encapsulation of the nanoparticles were also noted to significantly decrease the number of cytokines induced (Ferrari et al. 2018).

Research undertaken by Heidegger et al. (Heidegger et al. 2016) detailed the effects of mesoporous silica nanoparticles on immune cells (macrophages, lymphocytes, leukocytes). Results of this study detailed that the silica nanoparticles displayed minimal surface expression of activation markers and release of pro-inflammatory cytokines. Additionally, when the mesoporous silica nanoparticles were capped with a pH-responsive polymer and loaded with the Toll-like receptor 7 agonist R848, the immune-activating drug, a significant immune response was displayed.

6 Conclusion

Nanoparticulate delivery has considerably advanced the treatment of physiological conditions through enhancement of drug release, site targeting, and minimizing of drug dosing and side effects. The effects of these nanoparticles on serum constituents, however, are a significant parameter that has to be considered before the developed nanoparticles can be effectively used in patients. This will ultimately ensure that the nanosystem exerts the required properties intended with its administration.

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