Interaction between drug delivery vehicles and cells under the effect of shear stress

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Over the last decades, researchers have developed an ever greater and more ingenious variety of drug delivery vehicles (DDVs). This has made it possible to encapsulate a wide selection of therapeutic agents, ranging from proteins, enzymes, and peptides to hydrophilic and hydrophobic small drugs while, at the same time, allowing for drug release to be triggered through a diverse range of physical and chemical cues. While these advances are impressive, the field has been lacking behind in translating these systems into the clinic, mainly due to low predictability of *in vitro* and rodent *in vivo* models. An important factor within the complex and dynamic human *in vivo* environment is the shear flow observed within our circulatory system and many other tissues. Within this review, recent advances to leverage microfluidic devices to better mimic these conditions through novel *in vitro* assays are summarized. By grouping the discussion in three prominent classes of DDVs (lipidic and polymeric particles as well as inorganic nanoparticles), we hope to guide researchers within drug delivery into this exciting field and advance a further implementation of these assay systems within the development of DDVs. © *2015 AIP Publishing LLC*.
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I. INTRODUCTION

A plethora of drug delivery vehicles (DDVs) have been developed in the last decades and many of them have been successfully employed in *in vitro* studies. However, the translation of DDVs from *in vitro* to the preclinical and clinical stage has proven a considerable challenge and only few DDVs have reached the market.[1–3] An explanation for this fact is that many DDVs, which have shown remarkable efficacy in preclinical trials, almost completely lose their effectiveness when progressing to clinical studies. This serves as a striking example of the difficulties associated with predicting the behavior of a carrier in such a complex system as the human body.[4,5]

To date, researchers have relied on cell culture or animal models to study the potential of different DDVs. The step from a petri dish to an animal is quite large and, while cell cultures lack the complexity of biological tissues, direct *in situ* observation of carrier vehicles in animal models, apart from being prohibitively slow and expensive, are also very challenging due to practical challenges associated with visualization of particles in blood vessels and isolating contributions from numerous factors.

The discrepancy between the preclinical and clinical data indicates a need for the development and increased usage of new and improved *in vitro* testing assays that better mimic the environment of a human body.[6] Important factors affecting the behavior of DDVs in a biological system include both physicochemical parameters of the DDVs as well as characteristics of the biological target environment. Features of the biological target tissue that need to be taken

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into account when evaluating DDVs *in vitro* include their interaction with the rethiculo endothelial system, the extravasation of DDVs into highly permeable tumor tissues, the inability of two-dimensional cultures to reproduce extracellular barriers (which could be overcome by making use of multilayer cell cultures that incorporate multiple cell types), or the differences in cell phenotypes between cells cultured as monolayers compared to cells in native tissue. Although mimicking all these different conditions may seem an enormous challenge, being aware of the complexity of the human body can allow placing the focus where most impact can be made.[4,7–10] For example, since every intravenously administered DDV will be in contact not only with the blood components but also with the constituent flow-dynamic environment, microfluidic chips that can better simulate the dynamic environment of an *in vivo* situation should be used to test different DDVs. Traditional cell culture studies performed in well-plates under static conditions fail to consider the shear stress produced by the blood flow, which is generally known to affect a variety of cellular behaviors.[11–14] Additionally, the absence of flow can lead to the gravitational settling of the vehicles on the cell surface, which directly induces physiochemical stress in the cell. Furthermore, the appropriate cellular dosage of drug carriers can be misleading due to their settling or aggregation during static exposure conditions.[15]

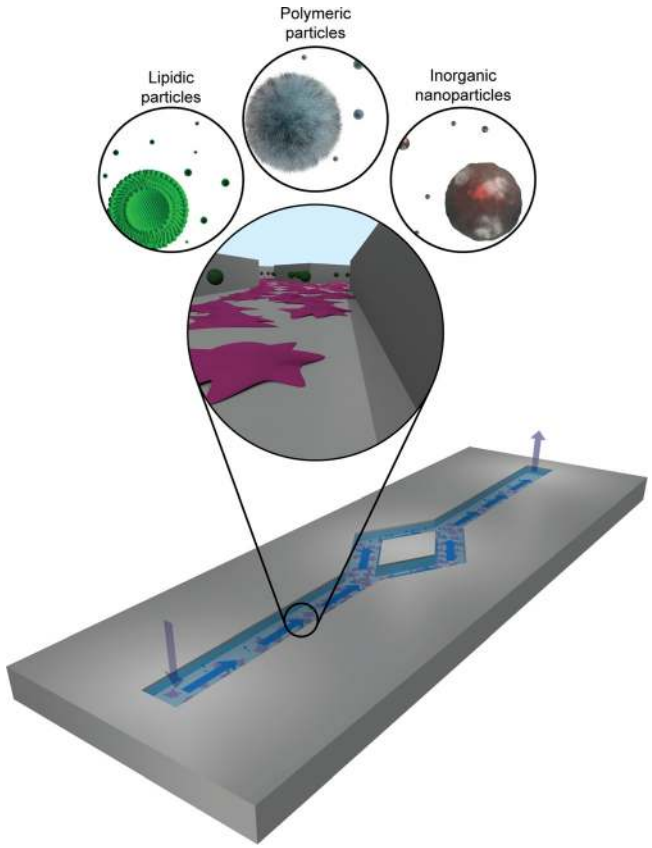
Microfluidic devices have started to emerge as a superior platform to test different DDVs since they can mimic the flow created by body fluids under a microscopic environment. This allows for the simulation of the fluidic aspects of *in vivo* situation with facile control over the flow rate on the cells surface, enabling detailed analysis of the mechanisms that govern the interactions of the drug carriers with the cells and, therefore, facilitating the correlation between *in vitro* and *in vivo* studies.[4,16] Assessing the interaction of drug carriers with cells while considering shear stress has already led to interesting findings, which are expected to facilitate a more accurate prediction of how the different DDVs will perform *in vivo*. Therefore, it will narrow down potential lead candidates prior to animal experiments which are often tied to both ethical and financial considerations.

This review features recent advances in the use of microfluidic devices as an alternative approach to evaluate some of the most prominent classes of DDVs reported to date, namely, lipidic, polymeric, and inorganic particles, by taking into consideration the dynamic flow condition of the body (Scheme 1). Table I depicts a summary of the different DDVs described in the review, the cell line employed and the flow conditions applied to study their interaction with cells as well as the outcome of the performed experiment.

II. LIPIDIC PARTICLES

Liposomes, which are spherical particles consisting of a lipid membrane(s) encapsulating an aqueous medium, have been in the drug delivery field as carriers of therapeutic molecules for more than three decades due to their potential to encapsulate both hydrophobic and hydrophilic molecules while protecting them from harsh conditions (e.g., pH changes, light, and enzymes), the simplicity of their assembly, their inherent low toxicity, and the possibility to modify their surfaces.[16,17] However, it has to be noted that, although research on liposomes as DDVs has been in progress for over 30 years, there are only a few commercialized liposome-based formulations for intravenous drug delivery (e.g., Doxil, a PEGylated liposomal formulation of doxorubicin).[18] This fact demonstrates that there is still considerable need to address the challenges involved in the design and characterization of liposomal therapeutics. In the latter case, it is of paramount importance to consider the dynamic *in vivo* environment in the body. In recent years, the importance of shear stress in the cellular uptake/association of liposomal drug delivery systems has started to be considered,[16] and in this section we will highlight some of the most recent examples.

Hosta-Rigau and Stadler were among the first to study the effect that physiological shear stress had on the uptake of several liposomal formulations by different cell lines.[19] The authors employed a myoblast cell line due to their mechanosensitivity and also as a model cancer cell line for drug delivery purposes since myoblasts are an immortalized cell line. By evaluating the myoblast cell response to zwitterionic, negatively and positively charged liposomes in the



SCHEME 1. Schematic illustration of the administration of the most prominent drug delivery vehicles reported to date (lipidic, polymeric, and inorganic particles) into a microfluidic device containing pre-seeded cells with the aim to study their interaction/uptake by cells under the influence of shear stress.

presence of low shear stress, they demonstrated that positively charged liposomes have an enhanced interaction with cells in the presence of shear stress as compared to static conditions. Two low shear stresses (0.0146 dyn cm^{-2} and 0.146 dyn cm^{-2}) were investigated to mimic the environment in certain tissues. In particular, cells different than endothelial cells (ECs) such as liver or tumor cells will experience a dynamic environment due to the bile flow[20] or the interstitial pressure in tumors,[21] respectively, which create lower shear forces than the ones in the microvasculature ($\tau \sim 3$–10 dyn cm^{-2}). The results demonstrate that there is no significant difference in the cell uptake/association for zwitterionic and negatively charged liposomes as a function of shear stress. However, when employing positively charged liposomes, the cell uptake/association increased ~8-fold in the presence of both investigated shear stresses as compared to static conditions. To assess if the higher cell uptake/association could be translated into a higher therapeutic response of the cells, the authors loaded the positively charged liposomes with the antitumor compound thiocoraline (TC).[22] The cells were exposed to this formulation at a shear stress of 0.146 dyn cm^{-2} and at static conditions, followed by the assessment of their cell viability. The results showed that when administering TC-loaded liposomes under static conditions the cell viability was reduced by ~45%. Application of shear stress drastically increased the effectiveness of the antitumor compound and the cellular viability was reduced by ~73%. This study serves as an initial demonstration of how the combination several factors determine the effect of shear stress on the DDVs-cell interaction. Only through the combination of a favorable surface charge and shear stress an enhanced cellular association is observed. This interplay in determining the effect of shear stress on DDV-cell interactions is a common theme observed throughout this review and will be discussed in greater detail where appropriate.

TABLE I. Summary of the different DDVs described throughout the review the cell line employed and the flow conditions applied to study their interaction with cells as well as the outcome of the performed experiment.

Author	Reference	Lipidic particles	Cell line	Flow conditions	Experimental outcome
Hosta-Rigau *et al.*	16	Negatively charged, positively charged, and zwitterionic liposomes	Myoblast	Shear stress = 0.146 dyn cm^{-2}	Increased cell uptake/association of positively charged liposomes vs. negatively charged and zwitterionic
Teo *et al.*	23	Stealth (PEGylated) liposomes	Myoblasts	Shear stress = 0.146 dyn cm^{-2}	No difference of cell uptake of PEGylated liposomes vs. non-PEGylated
			Hepatocytes		Lower cellular uptake of PEGylated liposomes vs. non-PEGylated liposomes
Panneerselvam *et al.*	33	Polydopamine (PDA) and highly branched (HB)- pNiPAAM functionalized liposomes	Macrophages	Shear stress = 4 dyn cm^{-2}	Increased cell uptake/association of HB-pNiPAAM functionalized liposomes vs. PDA coated liposomes
Paulis *et al.*	40	Gd-based liposomal contrast agent functionalized with anti-ICAM-1 ($L_{aICAM-1}$)	Vascular endothelial cells (ECs)	Shear stress = 0.25 and 0.5 Pa	Decreased cell uptake/association of $L_{aICAM-1}$ when increasing shear stress
Author	Reference	Polymeric particles	Cell line	Flow conditions	Experimental outcome
Farokhzad *et al.*	53	PEGylated poly(lactic acid) (PLA) NPs and microparticles (MPs) conjugated to aptamers that recognize the prostate specific membrane antigen (PSMA) protein	Prostate cancer epithelial cell LNCaP (PSMA expressing)	Shear stress = 1 and 4.25 dyn cm^{-2}	NPs-aptamer adheres to LNCap at low shear stress NPs-aptamer does not adhere to LNCap at high shear stress MPs-aptamer does not adhere to LNCap at low shear stress
			Prostate cancer epithelial cells PC3 (PSMA non-expressing)		NPs-aptamer does not adhere to PC3 at low shear stress
Fillafer *et al.*	58	Poly(*D*, *L*-lactide-*co*-glycolide) (PLGA) MPs conjugated to protein wheat germ agglutinin (WGA)	Vascular ECs	Shear stress = 0.2 and 1 s^{-1}	Decrease of MPs-WGA binding to cells upon increasing shear stress
Kona *et al.*	61	PLGA NPs conjugated to glycocalicin (GPIbα) expressed in platelets	Vascular ECs	Shear stress = 20 dyn cm^{-2}	Increasing shear stress increased NPs-GPIb α adhesion
Kolhar *et al.*	72	Polystyrene (PS) NPs and rod-shaped NPs (nanorods) coated with aICAM-1 antibody (aICAM-1-mAb)	Vascular ECs	Shear stress = 15 and 250 s^{-1}	ICAM-1-mAb-coated nanorods exhibited the highest attachment to cell under shear stress

TABLE I. (*Continued.*)

Author	Reference	Lipidic particles	Cell line	Flow conditions	Experimental outcome
Bhowmick *et al.*	74	aICAM-1-mAb PS-NPs	Flow-adapted vascular ECs	Shear stress = 4 dyn cm^{-2}	
			Non-flow-adapted vascular ECs		Higher cell internalization under shear stress
Han *et al.*	75	PS-NPs conjugated to platelet-ECs adhesion molecule-1 (PECAM-1) antibodies (aPECAM-1-mAb)	Vascular ECs	Acute shear stress	Stimulated aPECAM-1-mAb-NPs internalization
				Chronic shear stress	Inhibited aPECAM-1-mAb-NPs internalization
Author	Reference	Inorganic particles	Cell line	Flow conditions	Experimental outcome
Samuel *et al.*	91	CdTe quantum dots (QDs)	ECs	Shear stress = 0.05, 0.1 and 0.5 Pa	Highest QDs uptake at lowest shear stress
Mahto *et al.*	15	QDs	Fibroblasts	Flow rate = 0.5 μl min^{-1}	Increased cell viability under shear stress conditions, indicating less toxicity
Albanese *et al.*	98	PEGylated gold NPs (AuNPs)	Melanocyte tumor-spheroid	Flow rate = 50 μl h^{-1}	Under shear stress AuNPs accumulation at the tissue periphery increases but penetration depth does not increase
Kim *et al.*	101	Sub-50 nm mesoporous silica (MS)-NPs	ECs	Shear stress = 0.5, 3.3, 6.2, and 6.6 N m^{-2}	Higher uptake/cell association under shear stress

A follow up work by the same group was performed by Teo *et al*. which explored the cell uptake of stealth liposomes in the presence of low shear stress.[23] A common strategy to increase the circulation time of liposomes by decreasing uptake by the reticuloendothelial system is PEGylation.[24,25] The authors employed an innovative approach to PEGylate positively charged liposomes by making use of poly(dopamine) (PDA) to coat them. PDA, which has recently attracted considerable interest for many biomedical applications,[26–29] was employed to coat liposomes since it can be further functionalized by thiols or amines in a straight forward manner (Figure 1(a-i)).[30] The PEGylation of the liposomes was subsequently performed employing a graft copolymer with a poly(*L*-lysine) (PLL) backbone with PEG side chains (PLL-*g*-PEG) (Figure 1(a-ii)). The cell uptake under the effect of shear stress of PEGylated liposomes (L_{PDA_PEG}) was studied. The immortalized myoblast cell line was employed as a model cancer cell line to assess the potential effect that the interstitial environment found in tumors can have on the cell/drug carrier interaction. The cell internalization of L_{PDA_PEG} was also investigated in hepatocytes due to their relevance in the liver. Unmodified drug carriers are removed from the blood system and end up predominantly in the spleen[31] or in the liver[32] where they are either internalized by Kupffer cells or transferred to the bile and removed by hepatocytes. An *in vitro* perfusion flow chamber with a monolayer of cultivated cells connected to a pumping system injecting a solution with fluorescently labelled liposomes was employed to conduct the experiments. The cells were then harvested and their fluorescence was measured by flow cytometry. The results show that under static conditions there is no significant difference in cellular uptake of PEGylated *vs*. non-PEGylated liposomes for both cell lines (i.e., myoblasts and hepatocytes) (Figures 1(b) and 1(c), respectively). However, when shear stress was applied, the results were different. For the myoblast cell line there was no significant difference in cell uptake/association when employing PEGylated or non-PEGylated liposomes under the effect of shear stress (Figure 1(b)). In contrast, when employing hepatocytes, there was a significantly higher cellular interaction/uptake with non-PEGylated liposomes than with the PEGylated ones under the presence of shear stress (Figure 1(c)). This clearly demonstrates that different cell types show markedly different responses to DDVs under the influence of shear stress, further adding to the complexity of predicting DDV-cell interactions and emphasizing the importance of utilizing microfluidic devices in DDV characterization.

In a subsequent study from the same group, surface-modifications beyond classical PEGylation were considered. Panneerselvam *et al*. employed PDA coated liposomes to post-functionalize them with two temperature responsive polymer derivatives of poly(*N*-isoporpylacrylamide) (pNiPAAm).[33] Aminated pNiPAAm (pNiPAAm-NH_2) and highly branched pNiPAAm (pNiPAAm-HB) were employed to coat the liposomes. Their internalization by macrophages under the influence of shear stress mimicking the dynamic *in vivo* conditions in the capillaries ($\tau_4 = 4\,\text{dyn cm}^{-2}$) was studied. While there was no significant difference in the cell uptake of the liposomes with the three different coatings under static conditions, when applying τ_4, liposomes coated with pNiPAAm-HB exhibited a significantly higher cell uptake after 2.5 h compared to liposomes coated with only PDA or with pNIPAAm-NH_2. This fact could be explained by the different ζ-potential of the coated liposomes. As expected, the more negatively charged carriers, PDA and pNiPAAm-NH_2 coated liposomes, had a reduced cell association/uptake. Similar to the study of Teo *et al*., this work also reveals the importance of surface modification in cellular uptake depending on the presence of shear stress.

When evaluating the interaction of DDVs with vascular ECs, considering the shear stress is central. The vascular endothelium plays an essential role in the regulation of the inflammatory phases of atherosclerosis, which is a generalized inflammatory process of the artery wall.[34–36] In response to local inflammatory stimuli, the expression of the endothelial intracellular adhesion molecule-1 (ICAM-1) is up-regulated. This effect is of major importance, since it allows for leukocyte adhesion, followed by their extravasation through the EC layer to the site of inflammation.[37,38] Non-invasive *in vivo* molecular imaging of endothelial ICAM-1 expression could provide valuable insights in the progression of cardiovascular disease-related inflammation, which could potentially improve diagnosis and treatment.[39] Paulis and co-workers presented a paramagnetic liposomal contrast agent for multimodal magnetic resonance imaging of

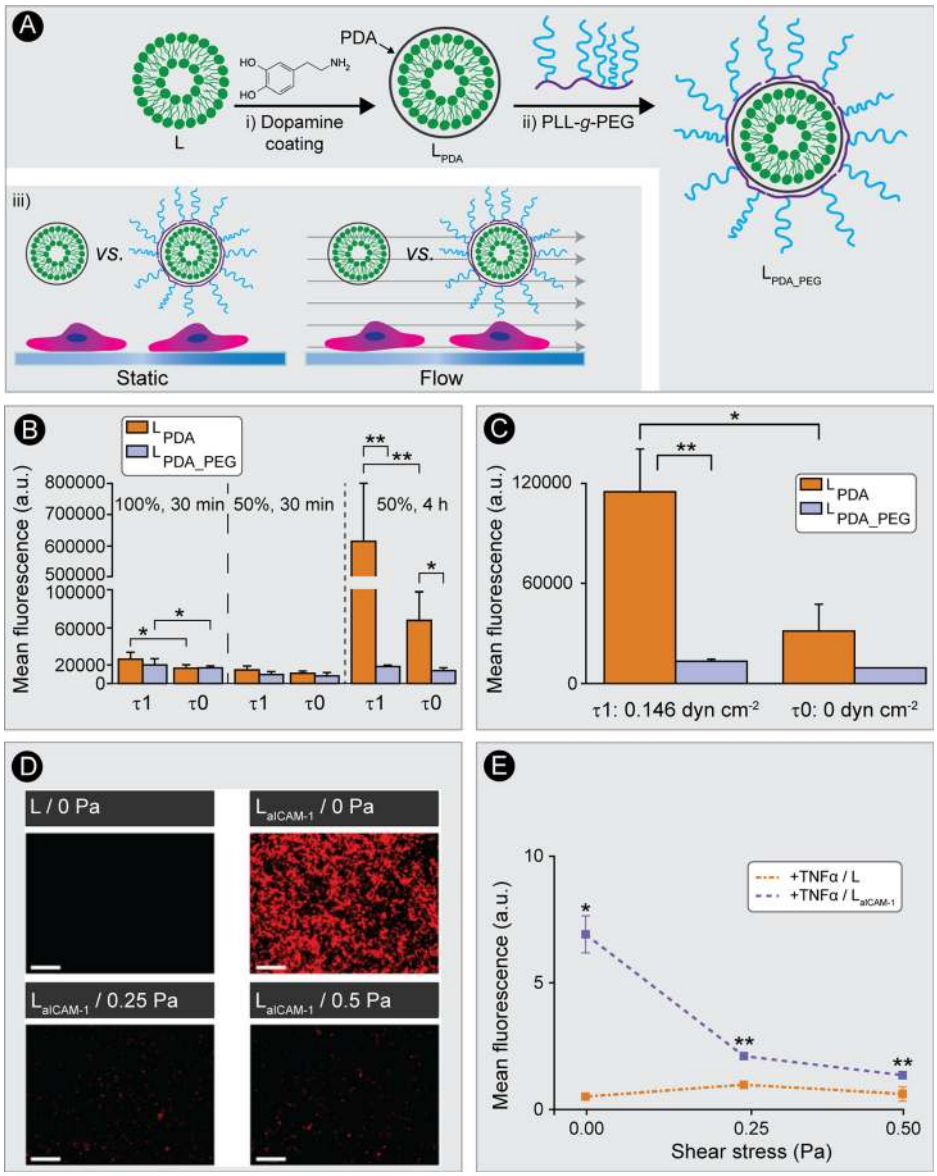


FIG. 1. (a) Schematic illustration of the coating process of liposomes and uptake experiments under static and flow conditions. (i) Liposomes are exposed to a dopamine solution for coating with PDA followed by the adsorption of PLL-g-PEG via Schiff Base addition and/or Michael addition of the amines of the PLL to the quinones of the PDA to form L_{PDA_PEG} (ii). Finally, the uptake/association efficiency of fluorescently labeled L_{PDA_PEG} hepatocytes and myoblasts under static and shear stress conditions was compared (iii). Reprinted with permission from Teo *et al.*, Biochim. Biophys. Acta, Gen. Subj. **1830**, 4838 (2013). Copyright 2013 Elsevier. (b) Mean fluorescence of myoblasts exposed to fluorescently labelled L_{PDA} and fluorescently labelled L_{PDA_PEG} (both either 100% (non-diluted) or 50% (2× diluted)) for 30 min and 4 h under static (τ_0) and shear stress (τ_1) conditions as assessed by flow cytometry Reprinted with permission from Teo *et al.*, Biochim. Biophys. Acta, Gen. Subj. **1830**, 4838 (2013). Copyright 2013 Elsevier. (c) Mean fluorescence of hepatocytes exposed to fluorescently labelled L_{PDA} and fluorescently labelled L_{PDA_PEG} for 30 min under static (τ_0) and shear stress ($\tau_1 = 0.146$ dyn cm^{-2}) conditions as assessed by flow cytometry. Reprinted with permission from Teo *et al.*, Biochim. Biophys. Acta, Gen. Subj. **1830**, 4838 (2013). Copyright 2013 Elsevier. (d) Fluorescence microscopy of ECs incubated with L or $L_{aICAM\text{-}1}$ containing rhodamine lipids (red) for 2 h at 37 °C and shear stress values of 0, 0.25 or 0.5 Pa. Scale bar = 100 μm. Reprinted with permission from Paulis *et al.*, J. Nanobiotechnol. **10**, 1 (2012). Copyright 2012 BioMed Central. (e) Cellular fluorescence levels quantified by flow cytometry at different shear stress levels. ECs were incubated with L or $L_{aICAM\text{-}1}$ containing rhodamine lipids (red) for 2 h at 37 °C and shear stress values of 0, 0.25, or 0.5 Pa. * = $p < 0.05$ vs. all groups, ANOVA with Bonferroni correction, ** = $p < 0.05$ vs. L_{IgG}, t-test. n = 2–5. Reprinted with permission from Paulis *et al.*, J. Nanobiotechnol. **10**, 1 (2012). Copyright 2012 BioMed Central.

endothelial ICAM-1 expression.[40] A Gd-based liposomal contrast agent was functionalized with anti-ICAM-1 (aICAM-1) antibodies ($L_{aICAM\text{-}1}$). The authors verified that the liposomal affinity for ICAM-1 was preserved in the presence of leukocytes and under physiological flow conditions, and that the efficiency of the *in vitro* binding of $L_{aICAM\text{-}1}$ to ICAM-1 was reduced with increasing shear stress within the physiological relevant range. As shown in Figure 1(d), fluorescence microscopy images of ECs incubated with rhodamine labelled L (0 Pa) and rhodamine labelled $L_{aICAM\text{-}1}$ (0, 0.25, and 0.5 Pa) show that the fluorescence signal resulting from $L_{aICAM\text{-}1}$ binding was detected at all applied shear stress values, whereas no significant fluorescence was observed after application of L. However, increasing shear stress resulted in a reduction of the fluorescence signal of $L_{aICAM\text{-}1}$, which indicates decreased binding to ICAM-1 as compared to static conditions (Figure 1(d)). After harvesting the cells from the flow chamber, the fluorescence intensity was quantified by flow cytometry and, as evidenced by a significant decrease in cellular fluorescence, the application of flow reduced the ability of $L_{aICAM\text{-}1}$ to adhere to ICAM-1 on ECs (Figure 1(e)). In contrast to the studies discussed previously, the DDVs in this work do not interact through a non-specific interaction (e.g., charge) but through a highly specific binding (i.e., antibody-antigen interaction). Interestingly, while in the former studies shear stress seemed to potentiate the DDV-cell interaction, herein, the DDVs bind less effectively with the cells, a fact that could be attributed to a potentially reduced surface residence time.

Taken together, these studies clearly indicate that when optimizing DDVs, taking into account the shear stress that the different DDVs will experience at the site of action is crucial, since the cell uptake will vary depending on parameters such as the carriers surface chemistry, the target cell type, and the DDVs mode of binding.

Holme *et al*. demonstrated the significance of shear stress to drug delivery by being the first to show shear-induced targeted drug delivery by engineering liposomes that are sensitive to mechanical stress.[41] While conventional spherical liposomes are highly stable to mechanical stress, lenticular, or lentil-shaped liposomes can suffer instabilities along their equator, which can turn into breaching points that lead to sensitivity to shear stress. Since, in atherosclerosis, the inflammatory process of the artery wall results in narrowing of arterial blood vessels, there are significant changes between the endogenous shear stress of healthy and constricted arteries.[42] The authors employed lenticular liposomes as a shear-induced drug delivery vehicle to constricted arteries. They designed an *in vitro* system containing either a healthy or a constricted poly(methyl methacrylate) artery model. An extracorporeal circulation pump was used to simulate the heart and to control the pressure and flow rate by regulating the pump speed. The authors showed that when employing dye-loaded lenticular liposomes, 17% of the entrapped dye was released in the constricted model while only 3.0% of dye release was observed after passing through the healthy artery model, therefore confirming the higher drug release under high shear stress conditions. This study presents an interesting proof-of-concept example that demonstrates that shear stress is not only important for our understanding of the behavior of DDVs *in vivo* but can also be utilized as a release trigger for controlled drug delivery. However, further studies are required to investigate the stability of these non-spherical liposomes in the highly varied *in vivo* environment.

III. POLYMERIC PARTICLES

Polymeric particles can be designed and engineered with a wide range of properties that can provide many improved ways for drug delivery applications, therefore offering several advantages over other types of particles. Polymeric particles have the ability to encapsulate a wide variety of drugs and release them over prolonged periods. Their surfaces are straight forward to modify with multiple targeting ligands able to interact with antigens that are differently expressed by a subset of cells or tissues, and they can also be fabricated from a wide range of materials that could be either degradable and/or possess an excellent stability, both *in vitro* and *in vivo*.[3] Also, central to the use of polymer particles for various applications is the optimization of parameters that influence cell-particle interactions. These parameters include size, chemical structure, and presence and density of escort molecules on the vehicle's surface, which

direct the targeted delivery. Recently, parameters such as the particles shape and mechanical properties in therapeutic functions have been highlighted.[3] Specifically, it has been shown that macrophages engulf rigid particles to a significantly higher extent than soft particles, a finding that has implications in immune clearance of nanoparticles (NPs). Recent studies have also shown that the shape of the particles can have an intriguing effect on their function,[43–48] and non-spherical nanoscale particles of various shapes, such as rods, discoids, or cubes, have been fabricated.[3,44,49–51] The common approach to evaluate cell-polymer particle interactions consists in performing sequential changes in the vehicle's design that affect each parameter, followed by the evaluation of the consequence of each change on the particles biodistribution using *in vivo* animal experiments. However, these studies may require a large number of experimental animals, in addition to being costly and time-consuming, thus imposing limits on the number of parameters that can be optimized and examined. In the study of polymer particle-cell interactions, microfluidics offer tools through which aspects of an *in vivo* environment can be mimicked, and responses to drug exposure studied.[4,52] In this section, we highlight several studies where microfluidic devices have been developed to mimic human (pathological) conditions to investigate the behavior of engineered polymer particles prior to *in vivo* animal experiments.

The Langer group was the first in studying the interaction of polymeric particles with cells under the presence of shear stress.[53] As a model system, they studied the interaction of targeted PEGylated poly(lactic acid) (PLA) NPs and microparticles (MPs) with two prostate cell lines that differ in their expression of the prostate specific membrane antigen (PSMA) protein. The PSMA protein is a well-known tumor antigen expressed on the surface of prostate cancer epithelial cells[54,55] as well as on the microvasculature of most studied tumors.[56] Therefore, vehicles that target the PSMA protein may have a broader utility in cancer therapy than just prostate cancer. The PEGylated PLA particles were conjugated to aptamers that recognize the PSMA protein[57] and exposed to the prostate cancer epithelial cells LNCaP or PC3 (PSMA expressing or non-expressing cell lines, respectively) under static conditions or under the effect of shear stress (Figure 2(a)). The (aptamer-conjugated) NPs were loaded with rhodamine-labeled dextran. The cell viability of the cells after being exposed to the different shear stress conditions was determined by staining the cell membrane with calcein AM (green) and the nuclei with DAPI (blue). The NP-aptamer bioconjugates are shown in red (Figure 2(b), top). The number of NPs or NPs-aptamer conjugate (NP + apt) attachment to LNCaP or PC3 cells was quantified under static or fluid flow conditions by manually counting the number of particles per cell (Figure 2(b), bottom). The results demonstrate that NP-aptamer bioconjugates selectively adhered to LNCaP but not to PC3 cells at static and low shear stress conditions ($\tau < 1$ dyn cm^{-2}) but they did not adhere when higher shear stress was applied ($\tau \sim 4.5$ dyn cm^{-2}). Control NPs and MPs lacking aptamers and MP-aptamer bioconjugates did not adhere to LNCaP cells, even under very low shear conditions ($\tau \sim 0.28$ dyn cm^{-2}). In accordance to what has been observed for lipidic particles, the presence of shear stress reduces the interaction between particle and cell if this interaction is highly specific (i.e., aptamer-antigen in this case). Interestingly, the authors found that this effect is further increased for larger particles, demonstrating that the presence of shear stress generated during fluid flow can have a significant effect on the binding characteristic of targeted delivery vehicles *in vitro*.

Studying the interaction of DDVs with human ECs under shear stress is of particular importance since, every intravenously administered DDV will travel in the blood circulation, being in contact with the constituent flow-dynamic environment and taken up by ECs forming the blood vessels. Fillafer *et al.* studied the interaction characteristics of protein-coated polymer MPs with human ECs under the influence of shear stress.[58] The cytoadhesive protein wheat germ agglutinin (WGA) was conjugated to poly(*D,L*-lactide-*co*-glycolide) (PLGA) MPs since several studies have shown that decoration with WGA mediates binding to the pharmaceutically relevant Caco-2 cell line under static conditions.[59,60] The targeting effect of WGA-MPs and MPs conjugated to the non-specific protein bovine serum albumin (BSA-MP) to ECs monolayers was investigated under static and low to moderate shear rates (between 0.2 s^{-1} and 1 s^{-1}). The results demonstrated that incubation at increasing flow velocities increasingly antagonized the attachment of both types of surface-modified particles. However, while binding of

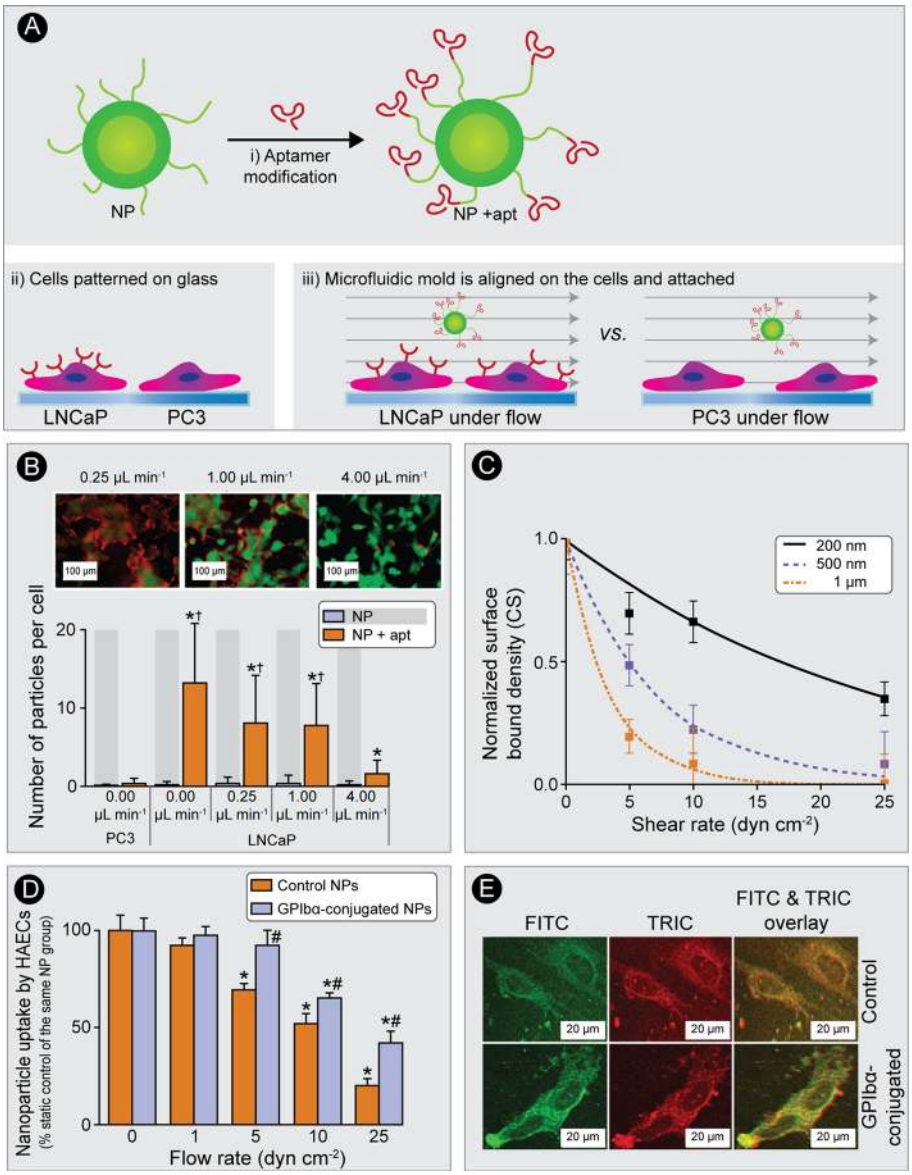


FIG. 2. (a) Schematic diagram of the PLA NPs-aptamer bioconjugate and the microfluidic device development. (i) The surface of PEGylated PLA particles was modified by covalent conjugation with RNA aptamers that recognize the PSMA protein. (ii) Model cell lines, LNCaP (+ PSMA) or PC3 (− PSMA), which differ in the pattern of PSMA expression, were patterned on glass substrates and (iii) a microfluidic mold was aligned over the cell pattern to develop the microchannels for experimental procedures. Reprinted with permission from Farokhzad *et al.*, Anal. Chem. **77**, 5453 (2005). Copyright 2005 American Chemical Society. (b) Fluorescent images of rhodamine-labeled dextran-encapsulated PLA NPs-aptamer bioconjugates binding to LNCaP cells under fluid flow at 0.25, 1, or 4 μl min $^{-1}$. The cell viability was determined by staining with calcein AM (green) and DAPI (blue), and the NPs-aptamer bioconjugates are shown in red. The number of NPs or NPs-aptamer bioconjugate (NP + apt) attachment to LNCaP or PC3 cells was quantified under static or fluid flow conditions by counting the number of particles per cell. An asterisk represents a statistically significant difference between NPs and NPs-aptamer bioconjugate groups ($p < 0.05$). A dagger represents a significant difference relative to the 4 μl min $^{-1}$ group ($p < 0.05$). Reprinted with permission from Farokhzad *et al.*, Anal. Chem. **77**, 5453 (2005). Copyright 2005 American Chemical Society. (c) Normalized NPs bound density at different shear stresses and size of the NPs. Reprinted with permission from Kona *et al.*, Int. J. Pharm. **423**, 516 (2012). Copyright 2012 Elsevier. (d) Adhesion and uptake of control NPs and GPIbα-NPs by ECs via measurement of NPs in lysis cell samples after 30 min of shear stress exposure. Asterisk indicates the significant differences compared to the same NPs group of static samples ($p < 0.05$). Hash key denotes the significant difference between GPIbα-NPs and control NPs ($p < 0.05$). Reprinted with permission from Kona *et al.*, Int. J. Pharm. **423**, 516 (2012). Copyright 2012 Elsevier. (e) Confocal microscopy images of ECs exposed to NPs and GPIbα-NPs. Fluorescent NPs were imaged using a FITC filter, while plasma membranes were stained with FM® 4-64 FX red membrane dye and imaged using a TRITC filter. Images on the right side represent the color overlay of FITC and TRITC filters. Reprinted with permission from Kona *et al.*, Int. J. Pharm. **423**, 516 (2012). Copyright 2012 Elsevier.

BSA-MPs was totally inhibited by shear stress, grafting with WGA resulted in a pronounced anchoring effect. While this study further emphasizes the ability of shear stress to reduce particle-cell interactions, it also demonstrates the importance of targeting in carriers to facilitate anchoring at the target site.

Kona *et al.* also employed PLGA NPs, targeted to injured arterial walls, to study their behavior under shear stress to evaluate their potential to treat cardiovascular pathological conditions.[61] Upon injury or under conditions like thrombosis, inflammation, and restenosis, the endothelium is activated and shows an increased expression of EC adhesion molecules such as P-selectin and E-selectin as compared to normal healthy cells.[62–65] The authors mimicked the natural binding ability of platelets to damaged ECs and functionalized PLGA NPs of several sizes with glycocalicin, the external fraction of glycoprotein Ib-α (GPIbα) of platelets, which has high affinity with P-selectin of damaged ECs as well as with the von Willebrand factor of the subendothelium. The results showed that increasing the shear stress to 20 dyn cm^{-2} decreased the cellular uptake of NPs by ECs by threefold as compared to the static conditions (Figure 2(c)). Such decrease in NPs adhesion at high shear stress levels and larger particle size may be explained by the larger dislodging forces under high shear stresses for bigger particles that might wash away adhered particles against adhesion. However, when the NPs were conjugated to GPIbα the results were different. They showed that conjugated GPIbα-NPs were taken up more by activated ECs under physiological flow conditions compared to their non-conjugated counterparts (Figures 2(d) and 2(e)). The authors speculate that this induced adhesion and uptake of GPIbα-NPs might be due to the higher binding strength of platelet ligand-GPIbα under high shear stress situations.[66,67] Interestingly, this study goes against the trend previously observed for targeted DDVs and highlights the importance of evaluating each individual system und flow. The influence of shear stress can often be difficult to predict due to a complex interplay of various factors.

The role of shape in vascular dynamics has long been known in terms of its influence on the behavior of circulatory cells such as erythrocytes and platelets,[68,69] and the shape of NPs in the circulation is of particular interest because it has a significant impact on hydrodynamics and interactions with vascular targets.[70,71] Kolhar *et al.* studied the targeting of ECs under shear stress while also taking into account the shape of the delivery system.[72] The authors employed polystyrene (PS) NPs and rod-shaped NPs (nanorods) coated with aICAM-1 antibodies (aICAM-1-mAb) and exposed them to rat brain ECs (RBE4) under flow at shear rates ranging from 15 to 250 s^{-1}. As controls for non-specific interactions, IgG-coated NPs were used. The results showed that ICAM-1-mAb-coated nanorods exhibited the highest attachment to the endothelial monolayer under flow conditions as compared to static conditions. ICAM-1-mAb-coated nanorods exhibited greater attachment/internalization to the endothelial monolayer under flow conditions than ICAM-1-mAb-coated NPs, IgG-coated nanorods, and IgG-coated NPs; thus confirming that endothelial targeting can be further enhanced by engineering the shape of ligand-displaying NPs. Additionally, *in vivo* experiments in mice confirmed that shape-induced enhancement of vascular targeting is also observed under physiological conditions in lungs and brain for NPs displaying aICAM-1-mAb. This study demonstrates that the shear stress generated from fluid flow can have a significant effect on the binding characteristic of DDVs of different shapes *in vitro*.

A factor that has not been discussed so far is the influence of shear stress on the cellular behavior itself. The blood vessel walls react to multiple chemical and mechanical stimuli in the flowing blood, the mechanical factors mainly being pressure and shear stress. The ECs of the vessel walls undergo significant rearrangements when cultured in a flow environment, which is more similar to the cell physiology *in vivo* as compared to the static cell culture environment.[73] Bhowmick *et al.* cultured ECs under physiological shear stress for 24 h, which resulted in flow adaptation: cell elongation and formation of actin stress fibers aligned to the flow direction and studied cell uptake under shear stress conditions of PS-NPs functionalized with aICAM-1-mAb.[74] Their results showed that flow-adapted cells internalized aICAM-1-mAb PS-NPs under flow, although at slower rate *vs.* non flow-adapted cells under static incubation. This fact demonstrates that to better mimic the *in vivo* situation it is not only important to administer the DDVs under flow conditions but also to grow the cells under flow. This is an important

observation as it demonstrates that *in vivo* experiments should be carefully designed to mimic physiological conditions from start to finish.

Han *et al*. also employed PS-NPs to target ECs.[75] Although ECs do not internalize antibodies to marker glycoprotein platelet-ECs adhesion molecule-1 (PECAM-1), multivalent NPs coated with anti-PECAM-1 antibodies (aPECAM-1-mAb) trigger a non-canonical vesicular uptake pathway known as CAM-endocytosis[76,77] which is, therefore, a useful target for endothelial drug delivery.[78] With the aim to understand the factors modulating this pathway, which will help to further optimize the design and use of therapeutics targeted to the endothelium, the authors studied the effects of acute and chronic shear stress on endothelial endocytosis of NPs targeted to PECAM-1, since this molecule is also implicated in the sensing of shear stress.[79] They employed PS-NPs coated with aPECAM-1-mAb and their results demonstrated that EC adaptation to chronic flow, manifested by cellular alignment with flow direction and formation of actin stress fibers, inhibited aPECAM-1-mAb-coated NPs endocytosis. Acute induction of actin stress fibers by thrombin also inhibited aPECAM-1-mAb NPs endocytosis, demonstrating that formation of actin stress fibers impedes the EC endocytic machinery. In contrast, acute flow without stress fiber formation stimulated the endocytosis of aPECAM-1-mAb NPs. Thus, these studies demonstrate the importance of the local flow microenvironment for NPs uptake by the endothelium and suggest that cell culture models of NPs uptake should reflect the microenvironment and phenotype of the target cells.

Presently, the target effect of site-specific drug delivery systems is by default determined by *in vitro* cell binding assays under static conditions. However, as shown in the aforementioned reports, regarding the extent and specificity of particle binding, clear discrepancies between the results obtained from static and more realistic dynamic models of the *in vivo* environment, can be observed. In particular, the presence of substantial hydrodynamic drag forces upon application *in vivo* is expected to explicitly affect the deposition characteristics of ligand-coated particles. To attain preferential binding of the carrier to the diseased tissue in these environments, the size and ligand coating density of the particles have to be adjusted according to the flow conditions as well as the expected receptor density and affinity at the target tissue.

Similar to the work by Holme *et al*., Korin *et al*. employed PLGA NPs under the effect of shear stress as an innovative approach to deliver drugs to obstructed blood vessels.[80] Inspired by the natural physical mechanism of platelet targeting in which circulating platelets are locally activated by high shear stress in the obstructed blood vessels causing them to rapidly adhere to the adjacent narrowed vessels,[81–83] the authors designed a thrombolytic delivery system that targets drugs selectively to sites of flow obstruction, thus concentrating the active drug in these regions. In particular, they used the high shear stress caused by vascular narrowing as a targeting mechanism. They created microscale aggregates of PLGA NPs coated with the thrombolytic drug tissue plasminogen activator (tPA). Upon being exposed to abnormally high shear stress, like the ones encountered in highly constricted arteries,[68,84,85] the micro-aggregates broke up into nanoscale components. By using a microfluidic model of vascular narrowing that was designed to mimic regions of blood vessels with 90% obstruction, the authors demonstrated that, upon breaking up, because of the smaller size of the NPs compared to the micro-aggregates, the shear-dispersed NPs adhere more efficiently to the surface of the blood vessel than the larger micro-aggregates. The results demonstrated that, upon infusing the tPA-loaded micro-aggregates at physiological flow rates through the clot-occluded microfluidic channels, the shear-dispersed tPA-coated NPs accumulated at the surface of the artificial emboli, progressively dissolving the clots and reducing their size. In contrast, treatment with soluble tPA at the same concentration and flow conditions had negligible effects. This study is another example of how through careful carrier design shear stress can be utilized to achieve a controlled drug delivery.

IV. INORGANIC PARTICLES

Inorganic NPs are attractive due to their unique physical functions, such as the bright light emission of quantum dots (QDs),[86] the optical and thermal properties of gold NPs (AuNPs),[87] and the modular distribution of nano-sized pores of mesoporous silica (MS) NPs.[88,89]

Since the vascular endothelium is a potential target for therapeutic intervention in diverse pathological processes including inflammation, atherosclerosis, and thrombosis, the interactions under shear stress of drug DDVs different than lipidic or polymeric particles, such as QDs, AuNPs, and MS-NPs, have also been studied.

QDs, which are semiconductor inorganic nanocrystals, have been found to be extremely useful for biological and medical applications due to their unique size-tunable optical and electronic properties such as tunable narrow emission spectra, high quantum yields, broad absorption spectra, and high resistance to photobleaching.[15,90,91] Samuel *et al.*[91] investigated the interactions of negatively charged CdTe QDs with ECs under static conditions or under low, medium, and high shear stress rates (0.05, 0.1, and 0.5 Pa, respectively) since, in humans, the mean shear stress varies between 0.05 and 0.76 Pa in the veins, 0.3 and 0.7 Pa in the peripheral arteries (e.g., the brachial artery and the femoral artery), and 1.0 and 1.5 Pa in the central arteries (e.g., the carotid artery).[91–95] Vascular inflammation and associated endothelial damage were simulated by treatment with tumor necrosis factor-α (TNF-α) or by compromising the cell membrane with the use of a low Triton X-100 concentration. AFM studies showed the combinatorial effects of shear stress and QDs on ECs morphology, such as membrane ruffling (i.e., a meshwork of newly polymerized actin filaments) and the development of stress fibers. The authors demonstrated that shear stress-induced membrane ruffling further mediates the uptake of QDs by human ECs. Their results indicate a maximal uptake of QDs at a shear stress rate of 0.05 Pa, while, under static conditions (i.e., 0 Pa) QDs did not show any cellular uptake. Additionally, EC exposure to mild detergents or TNF-α treatment had no significant effect on QD uptake, which could suggest that the shear stress-induced QD uptake was independent of adhesion molecules expressed by activated endothelium such as selectins E, L, and P or PECAM-1 and ICAM-1. The overall message of this study is that the combinatorial influence of factors such as NP surface characteristics, the presence or absence of shear stress, and the functional state and cytoskeletal rearrangements of the ECs determine the ultimate fate of NPs accumulation, which could have direct consequences for parenterally administered NP-conjugated drugs, and therefore *in vitro* studies considering shear stress will have to be conducted to obtain the desired *in vivo* effect.

On a different note, the cytotoxicity of QDs has been a subject of concern for many researchers which have argued against the extensive uses of QDs because they may also pose risks and toxicity to human health and environment under certain conditions.[96] Several studies have revealed cytotoxicity induced by QDs; however, those studies are entirely based on static exposure conditions.[15,97] Mahto and co-workers evaluated QD toxicity in fibroblast cells by using microfluidics technology.[15] The outcomes obtained from shear stress conditions were compared to those of the static conditions (Figure 3(a)). The results suggested noticeable differences in the number of detached and deformed cells as well as the viability percentages between the two different conditions. Although the cells showed similar morphological changes in both cases of exposure conditions, i.e., static and shear stress, the number of detached cells was significantly higher under static conditions than under the effect of shear stress after being incubated with QDs for 12 h (Figures 3(b) and 3(c)). The results showed a significant difference in the percentage of live cells between the two different exposure conditions at equal concentrations of QDs (∼30% and 75% in the cases of static and shear stress conditions, respectively) (Figures 3(d) and 3(e)). The authors related the possible causes of QDs-induced cytotoxicity irrespective of the types of exposure condition to the intracellular production of reactive oxygen species and cadmium release. The difference in cell viability depending on the exposure conditions was explained by the fact that under shear stress, the gravitational settling of particles could be avoided and the shear stress probably assisted in the homogeneous distribution of NPs in the culture medium during exposure time. Moreover, the shear stress exposure conditions resembled *in vivo* physiological conditions very closely; therefore, offering potential advantages for nanotoxicity research.

Albanese *et al.* were the first in employing microfluidic devices to study the role of drug carrier design in tumor transport followed by its validation with an animal model.[98] Although there have been significant advancements in the chemical synthesis and design of

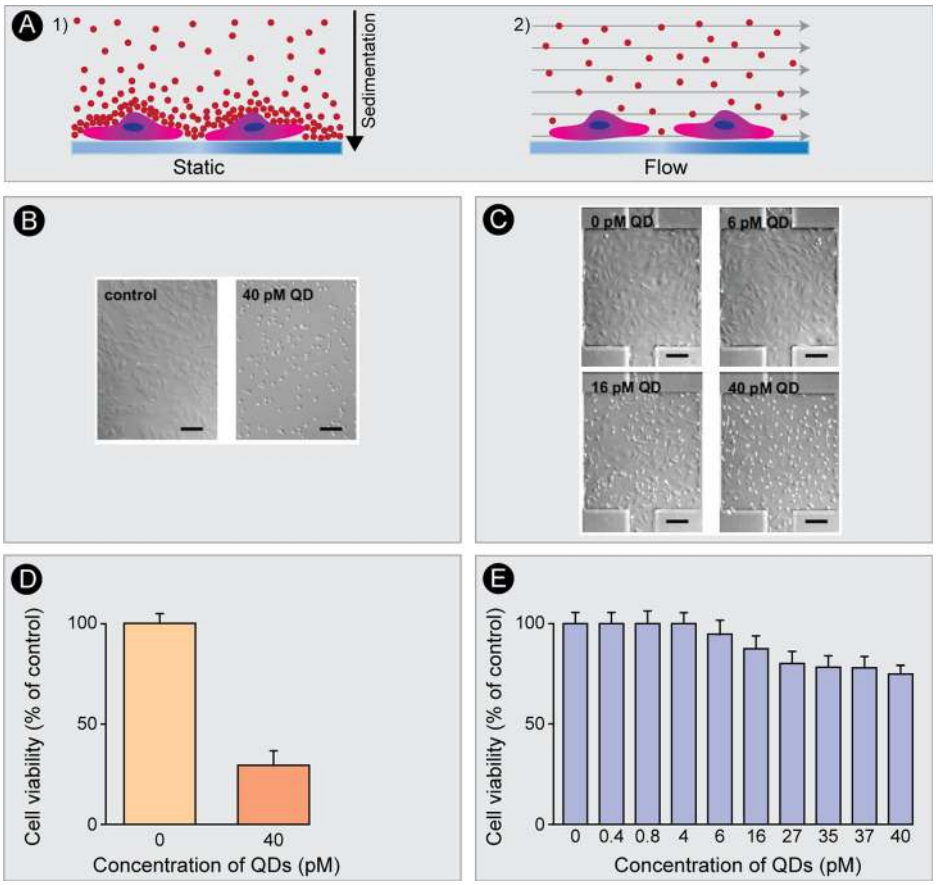


FIG. 3. (a) Schematic illustration of the static exposure condition (1), which shows the possibility of the sedimentation phenomenon in a conventional well-plate system, which can eventually lead to the non-homogeneous distribution of NPs, and consequently, the development of physicochemical stress on cells. (2) Schematic representation of the flow exposure condition in a microfluidic compartment that shows the homogeneous distribution of NPs. (b) Morphological effects of QDs in fibroblast cells under static and (c) flow exposure conditions. Scale bars = 100 μm. (d) Effects of QD on cell viability under static and (e) flow exposure conditions. Reprinted with permission from Mahto *et al.*, Biomicrofluidics **4**, 034111 (2010). Copyright 2010 AIP Publishing LLC.

cancer-targeting therapeutic and diagnostic agents, the delivery of these agents still remains inconsistent owing to poor control over pharmacokinetics and accumulation of carrier systems in diseased tissues *in vivo*.[98,99] Overcoming this problem requires a better understanding of how blood-borne carriers enter their target tissue and distribute at the cellular level. Albanese *et al.* employed a tumor-on-a-chip system where incorporation of tumor-like spheroids into a microfluidic channel permits real-time analysis of NP accumulation at physiological flow conditions.[98] The authors made use of this tumor-on-a-chip system to evaluate the influence of AuNP diameter, receptor targeting, and flow conditions on the transport of PEGylated AuNPs within a model tumor tissue.[100] They employed AuNPs since they can be prepared with precise control over their size and shape, which will in turn affect their optical and chemical properties, enhancing optical processes such as scattering, light absorption, fluorescence, and surface-enhanced Raman scattering.[87,89] The authors demonstrated that AuNP tissue accumulation was restricted to hydrodynamic diameters of $<$110 nm and that receptor targeting improved accumulation and retention. The flow rate applied to the AuNPs only affected their accumulation at the tissue periphery and did not increase their penetration depth. These findings were confirmed in a murine xenograft model, thus demonstrating that the tumor-on-a-chip provides an accurate *in vitro* method to predict *in vivo* AuNPs transport in murine tumor tissue.

The interaction of solid silica[19,91] and MS[91,101] NPs under the influence of shear stress has also been studied in several reports. Inorganic, biocompatible, porous ceramic NPs such as MS-NPs

which usually have a pore diameter of 2–4 nm, which can be tuned up to 30 nm, can be used for incorporating high levels of drugs and biomolecules. Drug loading can be further modulated by considering the interaction of the drugs with the walls of the pores. The surface chemistry of MS-NPs can also be modified for controlling their distribution after systemic administration.[89] MS-NPs possess additional properties such as water dispersity, resistance to microbial attack, and swelling, which make them attractive candidates for nanomedicine applications. Samuel *et al*. studied the internalization of 50 nm sized MS-NPs into ECs under the effect of shear stress, similar to the study of Mahto *et al*., demonstrating by AFM studies that the combinatorial effects of shear stress and NPs on ECs morphology, such as membrane ruffling and the development of stress fibers further mediates the uptake of MS-NPs. These results demonstrate that shear stress is critical for a realistic prediction of the uptake of inorganic particles.

Kim *et al*. studied the cytotoxicity of sub-50 nm MS-NPs to human ECs under microfluidic flow conditions.[101] The results demonstrated that unmodified MS-NPs show higher and shear stress-dependent toxicity to ECs under flow conditions. However, even under flow conditions, highly organo-modified MS-NPs show no significant toxicity to ECs.

V. OUTLOOK/FUTURE PERSPECTIVES

Human bodies are a highly complex system and possess a dynamic environment that represents a challenge for DDVs. By evaluating DDVs in classical *in vitro* assay systems, these conditions have been over simplified, leading to an overall low predictability towards their behavior in the clinic. The results discussed in this article demonstrate that a mentality shift is starting to occur in the drug delivery field with the realization that more predictive *in vitro* assay systems are necessary to progress DDVs towards the clinic. The discussed authors have shown that the shear stress observed within our blood as well as many tissues is an important factor that affects the interaction and impact DDVs have with and on cells. Microfluidic devices allow for the cultivation of cells and are ideal to mimic *in vivo* shear stress conditions.

The observed effects of shear stress can be traced back to three mechanisms: First, culturing cells under shear stress conditions induces changes of the cellular morphology, e.g., formation of stress fibers and membrane ruffling; second, shear stress causes changes in the interaction between DDVs and the cell due to, e.g., reduced binding strength due to the increased shear forces experienced by the DDV; and finally, the flow impacts the distribution of the DDVs in the culture medium, e.g., prevention of gravitational settling. As can be expected in systems that mimic complex *in vivo* environments, the overall effect observed on the cellular interaction of DDVs is a complex combination of these effects amongst others.

As such, it is difficult to draw specific conclusions on how shear stress affects the fate of drug carriers. Instead, they illustrate that shear stress is an important factor that should be taken into account both within carrier design and evaluation. The future of the field clearly lies within establishing procedures to implement microfluidic testing within the development of DDVs. For being able to retrieve reliable conclusions, a complete characterization of the physiochemical parameters of the different DDVs is required. However, altering one parameter, e.g., surface charge, while keeping the other parameters constant (hydrodynamic size, colloidal stability, nature of the coating, etc.) is not an easy endeavor. Furthermore, comparing effects throughout different studies is hampered by the fact that all the essential data on physicochemical characterization may not always be provided. Therefore, consensus should be reached on which parameters must be characterized as well as on the methods employed to perform the characterization. Additionally, the concentration of DDVs is another parameter on which consensus should be agreed upon. Although expressing the concentration in terms of mass per volume or particle number per volume, might be the easiest option, it is not always the most relevant, as smaller DDVs evoke different cell response than the larger ones at a similar mass per volume doses. Along with other groups,[102,103] we believe that the concentration should be expressed in terms of surface area per volume since both particle size and number are contained in this metric. Additionally, attention should also be paid to the standardization of assays and methods

in terms of incubation conditions such as the DDVs dose range as well as incubation time, which should mimic actual human exposure to DDVs. Furthermore, the results of these novel *in vitro* systems should be confirmed in humans and their greater predictability systematically demonstrated within a clinical setting. We predict that these future developments will put a strong toolset into the hands of researchers working on DDVs and will be an important contribution to the progression of the field towards the clinic.

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[1]A. Z. Wang, R. Langer, and O. C. Farokhzad, "Nanoparticle delivery of cancer drugs," Annu. Rev. Med. **63**, 185 (2012).
[2]E. K.-H. Chow and D. Ho, "Cancer nanomedicine: from drug delivery to imaging," Sci. Transl. Med. **5**, 1 (2013).
[3]G. Bao, S. Mitragotri, and S. Tong, "Multifunctional nanoparticles for drug delivery and molecular imaging," Annu. Rev. Biomed. Eng. **15**, 253 (2013).
[4]M. Bjoernmalm, Y. Yan, and F. Caruso, "Engineering and evaluating drug delivery particles in microfluidic devices," J. Controlled Release **190**, 139 (2014).
[5]D. L. Stirland, J. W. Nichols, S. Miura, and Y. H. Bae, "Mind the gap: A survey of how cancer drug carriers are susceptible to the gap between research and practice," J. Controlled Release **172**, 1045 (2013).
[6]J. Bowes, A. J. Brown, J. Hamon, W. Jarolimek, A. Sridhar, G. Waldron, and S. Whitebread, "Reducing safety-related drug attrition: The use of *in vitro* pharmacological profiling," Nat. Rev. Drug Discovery **11**, 909 (2012).
[7]K. Park, "Facing the truth about nanotechnology in drug delivery," ACS Nano **7**, 7442 (2013).
[8]P. Ruenraroengsak, J. M. Cook, and A. T. Florence, "Nanosystem drug targeting: Facing up to complex realities," J. Controlled Release **141**, 265 (2010).
[9]A. T. Florence, "'Targeting' nanoparticles: The constraints of physical laws and physical barriers," J. Controlled Release **164**, 115 (2012).
[10]T. Lammers, F. Kiessling, W. E. Hennink, and G. Storm, "Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress," J. Controlled Release **161**, 175 (2012).
[11]W. Yin, S. K. Shanmugavelayudam, and D. A. Rubenstein, "The effect of physiologically relevant dynamic shear stress on platelet and endothelial cell activation," Thromb. Res. **127**, 235 (2011).
[12]E. Tzima, M. A. Del Pozo, W. B. Kiosses, S. A. Mohamed, S. Li, S. Chien, and M. A. Schwartz, "Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression," EMBO J. **21**, 6791 (2002).
[13]P. F. Davies, A. Remuzzi, E. J. Gordon, C. F. Dewey, and M. A. Gimbrone, "Turbulent fluid shear stress induces vascular endothelial cell turnover *in vitro*," Proc. Natl. Acad. Sci. U.S.A. **83**, 2114 (1986).
[14]D. W. Dumbauld, H. Shin, N. D. Gallant, K. E. Michael, H. Radhakrishna, and A. J. García, "Contractility modulates cell adhesion strengthening through focal adhesion kinase and assembly of vinculin-containing focal adhesions," J. Cell. Physiol. **223**, 746 (2010).
[15]S. K. Mahto, T. H. Yoon, and S. W. Rhee, "A new perspective on *in vitro* assessment method for evaluating quantum dot toxicity by using microfluidics technology," Biomicrofluidics **4**, 034111 (2010).
[16]L. Hosta-Rigau, P. Schattling, B. M. Teo, M. E. Lynge, and B. Stadler, "Recent progress of liposomes in nanomedicine," J. Mater. Chem. B **2**, 6686 (2014).
[17]A. Musyanovych and K. Landfester, "Polymer micro- and nanocapsules as biological carriers with multifunctional properties," Macromol. Biosci. **14**, 458 (2014).
[18]Y. Barenholz, "Doxil®—the first FDA-approved nano-drug: Lessons learned," J. Controlled Release **160**, 117 (2012).
[19]L. Hosta-Rigau and B. Stadler, "Shear stress and its effect on the interaction of myoblast cells with nanosized drug delivery vehicles," Mol. Pharmaceutics **10**, 2707 (2013).
[20]T. Shimazu, *Liver Innervation: And the Neural Control of Hepatic Function* (John Libbey & Company Ltd, 1996).
[21]R. K. Jain, "Transport of molecules, particles, and cells in solid tumors," Annu. Rev. Biomed. Eng. **1**, 241 (1999).
[22]F. Romero, F. Espliego, J. P. Baz, T. G. DeQuesada, D. Gravalos, F. DelaCalle, and J. L. Fernández-Puentes, "Thiocoraline, a new depsipeptide with antitumor activity produced by a marine Micromonospora. I. Taxonomy, fermentation, isolation, and biological activities," J. Antibiot. **50**, 734 (1997).
[23]B. M. Teo, R. van der Westen, L. Hosta-Rigau, and B. Städler, "Cell response to PEGylated poly(dopamine) coated liposomes considering shear stress," Biochim. Biophys. Acta, Gen. Subj. **1830**, 4838 (2013).
[24]T. M. Allen, C. Hansen, and J. Rutledge, "Liposomes with prolonged circulation times: Factors affecting uptake by reticuloendothelial and other tissues," Biochim. Biophys. Acta **981**, 27 (1989).
[25]S. M. Moghimi and J. Szebeni, "Stealth liposomes and long circulating nanoparticles: Critical issues in pharmacokinetics, opsonization and protein-binding properties," Prog. Lipid Res. **42**, 463 (2003).
[26]S. H. Ku, J. Ryu, S. K. Hong, H. Lee, and C. B. Park, "General functionalization route for cell adhesion on non-wetting surfaces," Biomaterials **31**, 2535 (2010).
[27]Y. B. Lee, Y. M. Shin, J.-h. Lee, I. Jun, J. K. Kang, J.-C. Park, and H. Shin, "Polydopamine-mediated immobilization of multiple bioactive molecules for the development of functional vascular graft materials," Biomaterials **33**, 8343 (2012).
[28]K. Yang, J. S. Lee, J. Kim, Y. B. Lee, H. Shin, S. H. Um, J. B. Kim, K. I. Park, H. Lee, and S.-W. Cho, "Polydopamine-mediated surface modification of scaffold materials for human neural stem cell engineering," Biomaterials **33**, 6952 (2012).
[29]K. Kang, I. S. Choi, and Y. Nam, "A biofunctionalization scheme for neural interfaces using polydopamine polymer," Biomaterials **32**, 6374 (2011).

[30]M. E. Lynge, R. van der Westen, A. Postma, and B. Stadler, "Polydopamine-a nature-inspired polymer coating for biomedical science," Nanoscale **3**, 4916 (2011).
[31]S. M. Moghimi, "Mechanisms of splenic clearance of blood cells and particles: Towards development of new splenotropic agents," Adv. Drug Delivery Rev. **17**, 103 (1995).
[32]H. S. Choi, W. Liu, P. Misra, E. Tanaka, J. P. Zimmer, B. I. Ipe, M. G. Bawendi, and J. V. Frangioni, "Renal clearance of quantum dots," Nat. Biotechnol. **25**, 1165 (2007).
[33]K. Panneerselvam, S. Mena-Hernando, B. M. Teo, K. N. Goldie, and B. Stadler, "Liposomes equipped with poly(N-isopropyl acryl amide)-containing coatings as potential drug carriers," RSC Adv. **4**, 44769 (2014).
[34]C. K. Glass and J. L. Witztum, "Atherosclerosis: The road ahead," Cell **104**, 503 (2001).
[35]N. G. Frangogiannis, C. W. Smith, and M. L. Entman, "The inflammatory response in myocardial infarction," Cardiovasc. Res. **53**, 31 (2002).
[36]B. Rossi, S. Angiari, E. Zenaro, S. L. Budui, and G. Constantin, "Vascular inflammation in central nervous system diseases: Adhesion receptors controlling leukocyte-endothelial interactions," J. Leukocyte Biol. **89**, 539 (2011).
[37]S. A. Mousa, "Cell adhesion molecules: Potential therapeutic and diagnostic implications," Mol. Biotechnol. **38**, 33 (2008).
[38]C. Johnson-Leger and B. A. Imhof, "Forging the endothelium during inflammation: Pushing at a half-open door?," Cell Tissue Res. **314**, 93 (2003).
[39]F. Leuschner and M. Nahrendorf, "Molecular imaging of coronary atherosclerosis and myocardial infarction considerations for the bench and perspectives for the clinic," Circ. Res. **108**, 593 (2011).
[40]L. E. M. Paulis, I. Jacobs, N. M. van den Akker, T. Geelen, D. G. Molin, L. W. E. Starmans, K. Nicolay, and G. J. Strijkers, "Targeting of ICAM-1 on vascular endothelium under static and shear stress conditions using a liposomal Gd-based MRI contrast agent," J. Nanobiotechnol. **10**, 1 (2012).
[41]M. N. Holme, I. A. Fedotenko, D. Abegg, J. Althaus, L. Babel, F. Favarger, R. Reiter, R. Tanasescu, P.-L. Zaffalon, A. Ziegler, B. Mueller, T. Saxer, and A. Zumbuehl, "Shear-stress sensitive lenticular vesicles for targeted drug delivery," Nat. Nanotechnol. **7**, 536 (2012).
[42]R. de Crom, C. Cheng, F. Helderman, and R. Krams, "Large variations in absolute wall shear stress levels within one species and between species," Atherosclerosis **204**, 16 (2009).
[43]J. A. Champion, Y. K. Katare, and S. Mitragotri, "Particle shape: A new design parameter for micro- and nanoscale drug delivery carriers," J. Controlled Release **121**, 3 (2007).
[44]S. E. A. Gratton, P. A. Ropp, P. D. Pohlhaus, J. C. Luft, V. J. Madden, M. E. Napier, and J. M. DeSimone, "The effect of particle design on cellular internalization pathways," Proc. Natl. Acad. Sci. U.S.A. **105**, 11613 (2008).
[45]Y. Geng, P. Dalhaimer, S. S. Cai, R. Tsai, M. Tewari, T. Minko, and D. E. Discher, "Shape effects of filaments versus spherical particles in flow and drug delivery," Nat. Nanotechnol. **2**, 249 (2007).
[46]J. A. Champion and S. Mitragotri, "Role of target geometry in phagocytosis," Proc. Natl. Acad. Sci. U.S.A. **103**, 4930 (2006).
[47]J. A. Champion and S. Mitragotri, "Shape induced inhibition of phagocytosis of polymer particles," Pharm. Res. **26**, 244 (2009).
[48]P. Decuzzi, B. Godin, T. Tanaka, S. Y. Lee, C. Chiappini, X. Liu, and M. Ferrari, "Size and shape effects in the biodistribution of intravascularly injected particles," J. Controlled Release **141**, 320 (2010).
[49]J. A. Champion, Y. K. Katare, and S. Mitragotri, "Making polymeric micro- and nanoparticles of complex shapes," Proc. Natl. Acad. Sci. U.S.A. **104**, 11901 (2007).
[50]Y. D. Yin, Y. Lu, B. Gates, and Y. N. Xia, "Template-assisted self-assembly: A practical route to complex aggregates of monodispersed colloids with well-defined sizes, shapes, and structures," J. Am. Chem. Soc. **123**, 8718 (2001).
[51]D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, and P. S. Doyle, "Continuous-flow lithography for high-throughput microparticle synthesis," Nat. Mater. **5**, 365 (2006).
[52]K. Ziolkowska, R. Kwapiszewski, and Z. Brzozka, "Microfluidic devices as tools for mimicking the *in vivo* environment," New J. Chem. **35**, 979 (2011).
[53]O. C. Farokhzad, A. Khademhosseini, S. Jon, A. Hermmann, J. Cheng, C. Chin, A. Kiselyuk, B. Teply, G. Eng, and R. Langer, "Microfluidic system for studying the interaction of nanoparticles and microparticles with cells," Anal. Chem. **77**, 5453 (2005).
[54]M. Kawakami and J. Nakayama, "Enhanced expression of prostate-specific membrane antigen gene in prostate cancer as revealed by *in situ* hybridization," Cancer Res. **57**, 2321 (1997).
[55]R. S. Israeli, C. T. Powell, J. G. Corr, W. R. Fair, and W. D. W. Heston, "Expression of the prostate-specific membrane antigen," Cancer Res. **54**, 1807 (1994).
[56]S. S. Chang, D. S. O'Keefe, D. J. Bacich, V. E. Reuter, W. D. W. Heston, and P. B. Gaudin, "Prostate-specific membrane antigen is produced in tumor-associated neovasculature," Clin. Cancer Res. **5**, 2674 (1999).
[57]O. C. Farokhzad, S. Y. Jon, A. Khademhosseini, T. N. T. Tran, D. A. LaVan, and R. Langer, "Nanopartide-aptamer bioconjugates: A new approach for targeting prostate cancer cells," Cancer Res. **64**, 7668 (2004).
[58]C. Fillafer, G. Ratzinger, J. Neumann, Z. Guttenberg, S. Dissauer, I. K. Lichtscheidl, M. Wirth, F. Gabor, and M. F. Schneider, "An acoustically-driven biochip—impact of flow on the cell-association of targeted drug carriers," Lab Chip **9**, 2782 (2009).
[59]C. Fillafer, D. S. Friedl, M. Wirth, and F. Gabor, "Fluorescent bionanoprobes to characterize cytoadhesion and cytoinvasion," Small **4**, 627 (2008).
[60]Y. Mo and L.-Y. Lim, "Preparation and *in vitro* anticancer activity of wheat germ agglutinin (WGA)-conjugated PLGA nanoparticles loaded with paclitaxel and isopropyl myristate," J. Controlled Release **107**, 30 (2005).
[61]S. Kona, J.-F. Dong, Y. Liu, J. Tan, and K. T. Nguyen, "Biodegradable nanoparticles mimicking platelet binding as a targeted and controlled drug delivery system," Int. J. Pharm. **423**, 516 (2012).
[62]A. O. Eniola and D. A. Hammer, "Characterization of biodegradable drug delivery vehicles with the adhesive properties of leukocytes. II. Effect of degradation on targeting activity," Biomaterials **26**, 661 (2005).
[63]B. C. H. Lutters, M. A. Leeuwenburgh, C. C. M. Appeldoorn, T. J. M. Molenaar, T. J. C. Van Berkel, and E. A. L. Biessen, "Blocking endothelial adhesion molecules: A potential therapeutic strategy to combat atherogenesis," Curr. Opin. Lipidol. **15**, 545 (2004).

[64]X. Y. Zou, V. R. S. Patil, N. M. Dagia, L. A. Smith, M. J. Wargo, K. A. Interliggi, C. M. Lloyd, D. F. J. Tees, B. Walcheck, M. B. Lawrence, and D. J. Goetz, "PSGL-1 derived from human neutrophils is a high-efficiency ligand for endothelium-expressed E-selectin under flow," Am. J. Physiol. **289**, C415 (2005).
[65]H. S. Sakhalkar, J. Hanes, J. Fu, U. Benavides, R. Malgor, C. L. Borruso, L. D. Kohn, D. T. Kujiaka, and D. J. Goetz, "Enhanced adhesion of ligand-conjugated biodegradable particles to colitic venules," FASEB J. **19**, 792 (2005).
[66]J. Chen and J. A. López, "Interactions of platelets with subendothelium and endothelium," Microcirculation **12**, 235 (2005).
[67]R. K. Andrews, Y. Shen, E. E. Gardiner, and M. C. Berndt, "Platelet adhesion receptors and (patho)physiological thrombus formation," Histol. Histopathol. **16**, 969 (2001).
[68]D. M. Wootton and D. N. Ku, "Fluid mechanics of vascular systems, diseases, and thrombosis," Annu. Rev. Biomed. Eng. **1**, 299 (1999).
[69]R. T. Schoephoerster, F. Oynes, G. Nunez, M. Kapadvanjwala, and M. K. Dewanjee, "Effects of local geometry and fluid-dynamics on regional platelet deposition on artificial surfaces," Arterioscler. Thromb. **13**, 1806 (1993).
[70]S.-Y. Lee, M. Ferrari, and P. Decuzzi, "Shaping nano-/micro-particles for enhanced vascular interaction in laminar flows," Nanotechnology **20**, 495101 (2009).
[71]N. Doshi, B. Prabhakarpandian, A. Rea-Ramsey, K. Pant, S. Sundaram, and S. Mitragotri, "Flow and adhesion of drug carriers in blood vessels depend on their shape: A study using model synthetic microvascular networks," J. Controlled Release **146**, 196 (2010).
[72]P. Kolhar, A. C. Anselmo, V. Gupta, K. Pant, B. Prabhakarpandian, E. Ruoslahti, and S. Mitragotri, "Using shape effects to target antibody-coated nanoparticles to lung and brain endothelium," Proc. Natl. Acad. Sci. U.S.A. **110**, 10753 (2013).
[73]P. F. Davies, J. A. Spaan, and R. Krams, "Shear stress biology of the endothelium," Ann. Biomed. Eng. **33**, 1714 (2005).
[74]T. Bhowmick, E. Berk, X. Cui, V. R. Muzykantov, and S. Muro, "Effect of flow on endothelial endocytosis of nanocarriers targeted to ICAM-1," J. Controlled Release **157**, 485 (2012).
[75]J. Han, B. J. Zern, V. V. Shuvaev, P. F. Davies, S. Muro, and V. Muzykantov, "Acute and chronic shear stress differently regulate endothelial internalization of nanocarriers targeted to platelet-endothelial cell adhesion molecule-1," ACS Nano **6**, 8824 (2012).
[76]V. R. Muzykantov, M. Christofidou-Solomidou, I. Balyasnikova, D. W. Harshaw, L. Schultz, A. B. Fisher, and S. M. Albelda, "Streptavidin facilitates internalization and pulmonary targeting of an anti-endothelial cell antibody (platelet-endothelial cell adhesion molecule 1): A strategy for vascular immunotargeting of drugs," Proc. Natl. Acad. Sci. U.S.A. **96**, 2379 (1999).
[77]S. Muro, R. Wiewrodt, A. Thomas, L. Koniaris, S. M. Albelda, V. R. Muzykantov, and M. Koval, "A novel endocytic pathway induced by clustering endothelial ICAM-1 or PECAM-1," J. Cell Sci. **116**, 1599 (2003).
[78]E. Simone, B.-S. Ding, and V. Muzykantov, "Targeted delivery of therapeutics to endothelium," Cell Tissue Res. **335**, 283 (2009).
[79]K. Fujiwara, "Platelet endothelial cell adhesion molecule-1 and mechanotransduction in vascular endothelial cells," J. Intern. Med. **259**, 373 (2006).
[80]N. Korin, M. Kanapathipillai, B. D. Matthews, M. Crescente, A. Brill, T. Mammoto, K. Ghosh, S. Jurek, S. A. Bencherif, D. Bhatta, A. U. Coskun, C. L. Feldman, D. D. Wagner, and D. E. Ingber, "Shear-activated nanotherapeutics for drug targeting to obstructed blood vessels," Science **337**, 738 (2012).
[81]Z. M. Ruggeri, J. N. Orje, R. Habermann, A. B. Federici, and A. J. Reininger, "Activation-independent platelet adhesion and aggregation under elevated shear stress," Blood **108**, 1903 (2006).
[82]W. S. Nesbitt, E. Westein, F. J. Tovar-Lopez, E. Tolouei, A. Mitchell, J. Fu, J. Carberry, A. Fouras, and S. P. Jackson, "A shear gradient-dependent platelet aggregation mechanism drives thrombus formation," Nat. Med. **15**, 665 (2009).
[83]S. Goto, H. Sakai, M. Goto, M. Ono, Y. Ikeda, S. Handa, and Z. M. Ruggeri, "Enhanced shear-induced platelet aggregation in acute myocardial infarction," Circulation **99**, 608 (1999).
[84]J. Strony, A. Beaudoin, D. Brands, and B. Adelman, "Analysis of shear stress and hemodynamic factors in a model of coronary artery stenosis and thrombosis," Am. J. Physiol. **265**, H1787 (1993).
[85]D. L. Bark and D. N. Ku, "Wall shear over high degree stenoses pertinent to atherothrombosis," J. Biomech. **43**, 2970 (2010).
[86]A. P. Alivisatos, "Semiconductor clusters, nanocrystals, and quantum dots," Science **271**, 933 (1996).
[87]M. C. Daniel and D. Astruc, "Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology," Chem. Rev. **104**, 293 (2004).
[88]I. I. Slowing, J. L. Vivero-Escoto, C.-W. Wu, and V. S. Y. Lin, "Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers," Adv. Drug Delivery Rev. **60**, 1278 (2008).
[89]H. Cabral, K. Miyata, and A. Kishimura, "Nanodevices for studying nano-pathophysiology," Adv. Drug Delivery Rev. **74**, 35 (2014).
[90]M. Yan, Y. Zhang, K. Xu, T. Fu, H. Qin, and X. Zheng, "An *in vitro* study of vascular endothelial toxicity of CdTe quantum dots," Toxicology **282**, 94 (2011).
[91]S. P. Samuel, N. Jain, F. O'Dowd, T. Paul, D. Kashanin, V. A. Gerard, Y. K. Gun'ko, A. Prina-Mello, and Y. Volkov, "Multifactorial determinants that govern nanoparticle uptake by human endothelial cells under flow," Int. J. Nanomed. **7**, 2943 (2012).
[92]M. Kroll, J. Hellums, L. McIntire, A. Schafer, and J. Moake, "Platelets and shear stress," Blood **88**, 1525 (1996).
[93]R. Reneman and A. G. Hoeks, "Wall shear stress as measured *in vivo*: Consequences for the design of the arterial system," Med. Biol. Eng. Comput. **46**, 499 (2008).
[94]P. V. Stroev, P. R. Hoskins, and W. J. Easson, "Distribution of wall shear rate throughout the arterial tree: A case study," Atherosclerosis **191**, 276 (2007).
[95]S. P. Wu, S. Ringgaard, S. Oyre, M. S. Hansen, S. Rasmus, and E. M. Pedersen, "Wall shear rates differ between the normal carotid, femoral, and brachial arteries: An *in vivo* MRI study," J. Magn. Reson. Imaging **19**, 188 (2004).
[96]A. M. Derfus, W. C. W. Chan, and S. N. Bhatia, "Probing the cytotoxicity of semiconductor quantum dots," Nano Lett. **4**, 11 (2004).

[97]J. Lovrić, H. Bazzi, Y. Cuie, G. A. Fortin, F. Winnik, and D. Maysinger, "Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots," J. Mol. Med. **83**, 377 (2005).
[98]A. Albanese, A. K. Lam, E. A. Sykes, J. V. Rocheleau, and W. C. W. Chan, "Tumour-on-a-chip provides an optical window into nanoparticle tissue transport," Nat. Commun. **4**, 2718 (2013).
[99]M. E. Davis, Z. Chen, and D. M. Shin, "Nanoparticle therapeutics: An emerging treatment modality for cancer," Nat. Rev. Drug Discovery **7**, 771 (2008).
[100]L. Y. T. Chou and W. C. W. Chan, "Fluorescence-tagged gold nanoparticles for rapidly characterizing the size-dependent biodistribution in tumor models," Adv. Healthcare Mater. **1**, 714 (2012).
[101]D. Kim, Y.-S. Lin, and C. L. Haynes, "On-chip evaluation of shear stress effect on cytotoxicity of mesoporous silica nanoparticles," Anal. Chem. **83**, 8377 (2011).
[102]F. Joris, B. B. Manshian, K. Peynshaert, S. C. De Smedt, K. Braeckmans, and S. J. Soenen, "Assessing nanoparticle toxicity in cell-based assays: Influence of cell culture parameters and optimized models for bridging the *in vitro-in vivo* gap," Chem. Soc. Rev. **42**, 8339 (2013).
[103]P. Rivera-Gil, D. Jimenez De Aberasturi, V. Wulf, B. Pelaz, P. Del Pino, Y. Zhao, J. M. De La Fuente, I. Ruiz De Larramendi, T. Rojo, X.-J. Liang, and W. J. Parak, "The challenge to relate the physicochemical properties of colloidal nanoparticles to their cytotoxicity," Acc. Chem. Res. **46**, 743 (2013).