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Review Article

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Particle Margination and Its Implications on Intravenous Anticancer Drug Delivery

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Abstract. "Margination" refers to the movement of particles in flow toward the walls of a channel. The term was first coined in physiology for describing the behavior of white blood cells (WBCs) and platelets in blood flow. The margination of particles is desirable for anticancer drug delivery because it results in the close proximity of drug-carrying particles to the endothelium, where they can easily diffuse into cancerous tumors through the leaky vasculature. Understanding the fundamentals of margination may further lead to the rational design of particles and allow for more specific delivery of anticancer drugs into tumors, thereby increasing patient comfort during cancer treatment. This paper reviews existing theoretical and experimental studies that focus on understanding margination. Margination is a complex phenomenon that depends on the interplay between inertial, hydrodynamic, electrostatic, lift, van der Waals, and Brownian forces. Parameters that have been explored thus far include the particle size, shape, density, stiffness, shear rate, and the concentration and aggregation state of red blood cells (RBCs). Many studies suggested that there exists an optimal particle size for margination to occur, and that nonspherical particles tend to marginate better than spherical particles. There are, however, conflicting views on the effects of particle density, stiffness, shear rate, and RBCs. The limitations of using the adhesion of particles to the channel walls in order to quantify margination propensity are explained, and some outstanding questions for future research are highlighted.

KEY WORDS: blood; cancer; margination; nanocarriers; nanoparticles.

INTRODUCTION

Margination is defined as the movement of particles in flow toward the walls of a channel. The margination of particles in blood has applications in microfluidic devices for the removal of pathogens and separation of cells (1–4). Margination is especially relevant to cancer diagnostics and therapy wherein it can bring sensing, imaging, and/or therapeutic nanoparticles closer to capillary entrances, which are located near the periphery of the larger blood vessels, thereby allowing these nanoparticles to enter the microcirculation more readily (5). Margination also allows the particles to come close to the endothelium and, by attaching biorecognition molecules such as antibodies to nanoparticles, the nanoparticles can then be used to sense the increased amount of integrins and receptors of tumor endothelial cells (6). In terms of therapeutics, margination enhances the diffusion of drug-carrying nanoparticles into the tumor sites through the enhanced permeability and retention (EPR) effect that results from the characteristic leaky vasculature and lack of lymphatic vessels near tumor (7). For some bioimaging applications, low or no margination propensity may be desirable so that the nanoparticles will circulate in the blood stream for an extended period of time (6). Nevertheless, understanding the fundamentals of margination is vital in order to engineer nanoparticles for more effective diagnostics and drug delivery. In this review paper, we will first describe the historical and theoretical background of margination. We will then focus on the factors that are known to affect margination. Outstanding questions and contradicting views related to the fundamental understanding of margination will be highlighted, and future perspectives on margination research will be presented at the end of this review. For a more in-depth discussion on the challenges of modeling margination, the readers are encouraged to read a recent review paper by Kumar and Graham (8).

HISTORICAL BACKGROUND

In physiology, margination refers to the migration of white blood cells (WBCs) toward the endothelium during

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blood flow and is relevant to the process of inflammation. Margination and the subsequent adhesion of WBCs to the endothelium allow the WBCs to transmigrate across the endothelial wall and enter an inflamed area of tissue, as seen in Fig. 1 (9). The margination of WBCs was first observed in the blood vessels of tadpole tails by Dutrochet in 1824 (10). It was again observed by Gert Vejlens in 1938, and the margination of WBCs was correlated with the aggregation of red blood cells (RBCs) *in vivo* (11). WBC margination is often followed by: (1) their adhesion to blood vessel walls, (2) the movement of the WBCs into the space between the endothelial cells, (3) the transport of WBCs through tissue, and (4) the treatment of inflammation by the WBCs. The steps following the margination of WBCs are fairly well understood, but the origin and detailed mechanism of margination remain unclear (12). While WBCs were the first "particles" that were observed to display the propensity to marginate, other particles, such as platelets and nanoparticles, have also been observed to exhibit margination in blood flow (13–15). One of the first mentions of particle margination is by Segré and Silberberg in 1962 during their studies of rigid spheres suspended in a mixture of glycerol, 1–3 butanediol, and water (16). The authors observed polymethylmethacrylate (PMMA) spheres with a radius ranging from 0.32 to 1.71 mm migrated away from the center of the tube and moved closer to the wall of the tube in Poiseuille flow (16).

PHYSICS OF MARGINATION

Inertial, Viscous, and Lift Forces

Blood flow in the body is generally considered as laminar flow, although the flow can become turbulent under certain conditions, such as in the ascending aorta (17). In laminar flow, a simple Newtonian fluid exhibits a parabolic velocity profile and the flow is composed of a multitude of fluid layers, oriented parallel to one another in the flow direction, that travel smoothly without disruption alongside one another. Unlike turbulent flow, in which fluid naturally experiences irregular disruptions that result in lateral mixing; there is no occurrence of lateral mixing from fluid convection in laminar flow. Laminar flow and turbulent flow are described by the dimensionless Reynolds number (*Re*), which is a ratio between the inertial and viscous forces acting on a flowing fluid. The *Re* is given by:

$$Re = \frac{\text{inertial forces}}{\text{viscous forces}} = \frac{\rho u L}{\eta} \tag{1}$$

where ρ is the density of the fluid, u is the average velocity of the fluid, L is the characteristic length of the fluid (e.g., the diameter of a channel or blood vessel), and η is the dynamic viscosity of the fluid. Turbulent flow occurs at high *Re* while laminar flow takes place at low *Re*. Recently, there has been a growing interest in exploiting fluid inertia in order to separate and concentrate particles. Interested readers may refer to a review paper by Di Carlo (18). Given the small diameter of many blood vessels in the human body, blood flow is usually classified as being laminar, although blood is a non-Newtonian fluid due to the presence of RBCs and other blood constituents.

It was first observed by Goldsmith *et al.* that RBCs formed an RBC-rich core region in the center of the flow in the vessel, which resulted in a cell-free layer (CFL) near the endothelium that was free of RBCs (19). The authors also found that WBCs added into glass tubes containing blood that was free of RBCs did not exhibit margination (19). The CFL is thin, approximately 3 μm thick for blood flow in a 40-μm diameter vessel, but its thickness varies with the vessel diameter and geometry (20). The geometric change near bifurcations, for example, changes the trajectory of RBCs and this geometry has been demonstrated to change the thickness of the CFL as a result of the nonuniform flow pattern (20). Furthermore, higher flow rates tend to break up RBC aggregates, thereby expanding the RBC-rich core and subsequently decreasing the thickness of the CFL (20).

An asymmetric pressure field may be developed beneath or above the blood cells as a result of lubricating flow between the wall and a blood cell, resulting in a "wall lift force" that pushes WBCs and RBCs away from the wall during fluid flow (21,22).This lift force can be calculated by:

$$F_l = \eta\dot{\gamma}\frac{R^3}{h} f(1-v) \tag{2}$$

where η is the viscosity of the medium, $\dot{\gamma}$ is the shear rate, R is the radius of the particle, h is the particle's distance from the wall and $f(1-v)$ is a dimensionless function (Fig. 2) (21,22). v is the reduced particle volume and is given by:

$$v = \frac{\mathrm{V}}{\frac{4}{3}\pi\left(\frac{S}{4\pi}\right)^{3/2}} \tag{3}$$

where V is the enclosed volume and S is the surface area of the particle (21,22). Generally, WBCs are stiffer than RBCs and maintain a roughly spherical shape even in high shear flow. The lift forces acting on WBCs are weak compared with those acting on RBCs, allowing WBCs to maintain their position in the CFL and remain close to the endothelium (21,23–25). Conversely, the discoidal shape of RBCs, combined with their

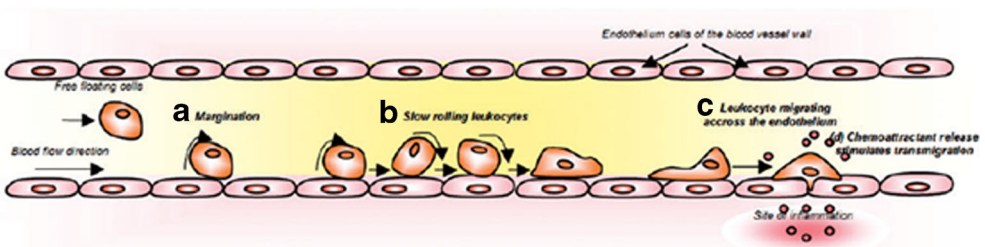

Fig. 1. Margination and transmembrane migration of a WBC to a site of inflammation (9). Reproduced from (9) with permission of The Royal Society of Chemistry (http://dx.doi.org/10.1039/B814567A)

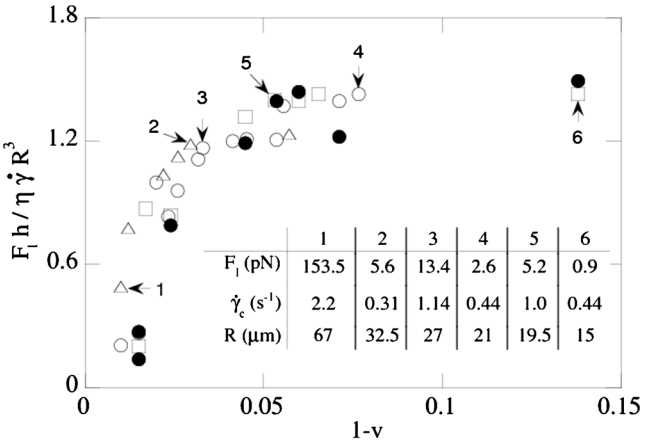

Fig. 2. Plot of particle reduced volume, from which the reduced volume function, f(*1−v*), can be calculated (22). Reprinted figure with permission from (22). Copyright 2002 by the American Physical Society (http://link.aps.org/doi/10.1103/PhysRevLett.88.068103)

elasticity and membrane fluidity, results in strong lift forces that push RBCs toward the center of the channel and lead to the formation of a CFL close to the endothelium (21,25). The formation of the CFL further leads to a decrease in the hydrodynamic resistance, which allows the WBCs to marginate more easily and reach the vessel wall (26,27). The deformability of the RBCs is crucial to their accumulation in the center of the vessel. In malaria patients, RBCs are significantly stiffer, and these infected RBCs have been shown to exhibit margination (3). Lift forces are generally thought to be responsible for the formation of a RBC-rich core region in the center of the blood vessels and for the margination of WBCs to the wall. However, according to Eq. 2, the actual lift forces acting on RBCs and WBCs also strongly depend on the actual cell size to the third power (22). The authors of this paper noted that in reference (21) WBCs were calculated to have a slightly larger lift force (46–230 pN) than the RBCs (31–155 pN) based on the blood cell sizes assumed.

Brownian Motion and Particle Adhesion

The Péclet number (*Pe*) is a dimensionless quantity that describes the mass transport by taking the ratio of the convective transport by the fluid motion and the diffusive transport by the chemical potential difference, mainly due to a concentration gradient (28). The *Pe* is given by (28):

$$Pe = \frac{\text{convective transport rate}}{\text{diffusive transport rate}} = \frac{Lu}{D} \tag{4}$$

where L is the characteristic length, u is the average velocity of the fluid, and D is the translational diffusion coefficient (28). Higher *Pe* indicate a larger hydrodynamic contribution to mixing whereas low *Pe* indicate a larger diffusional contribution. For a dilute suspension and a small *Re* number, the translational diffusion coefficient of a spherical particle can be calculated using the Stokes–Einstein–Sutherland equation (28):

$$D = \frac{k_B T}{6\pi\eta R} \tag{5}$$

where k_B is the Boltzmann constant, T is the temperature, η is the viscosity of the suspending medium, and R is the radius of the spherical particle. According to Eqs. 4 and 5, small particles have a higher diffusion coefficient and the corresponding *Pe* is smaller. This implies that diffusive transport becomes more significant in the case of nanoparticles. The end result is the random Brownian motion of nanoparticles, wherein the particles fluctuate across streamlines (29).

It should be noted that many studies use particle adhesion as a method of measurement for the magnitude of margination. These studies conjugated particles with a ligand and coated channels with a receptor or chose to model such a system in order to measure the margination of various particles (5,7,15,30–35). The conjugated particles became bound to the walls of the channels upon coming into contact via margination. In this way, margination was measureable based on the number of particles adhered to the walls. Particle adhesion and margination are closely related. However, particle adhesion is also affected by other factors such as hydrodynamic forces, the amount of ligands and receptors, the contact density, the characteristic length of the bond between the ligand and the receptor, the temperature of the medium, and particle deformability (30,35).

Intuitively, the contact area of a spherical particle with the adhesion layer is proportional to the particle radius, R, and the thickness of the adhesion layer. However, the drag force experienced by the larger particle also increases. For a spherical particle, the drag force is given as:

$$F_D = \frac{1}{2}\pi\rho U^2 C_d R^2 \tag{6}$$

where U is the velocity of the spherical particle relative to the fluid, and C_d is the drag coefficient. The value of C_d depends on the flow regimes. For small *Re* (calculated using the particle diameter as the characteristic length), the flow is in the Stokes regime wherein C_d scales with r^{-1}. As a result, the ratio of the adhesion to the drag forces would be independent of the size of the particle ($F_A/F_D \propto r/r$). For large *Re*, the flow is considered to be in the Newtonian regime, where C_d is a constant. Consequently, the ratio of the adhesion to the drag forces would scale with r^{-1}, implying that larger particles will detach from the adhesion layer eventually due to larger hydrodynamic drag. Such a simple scaling argument does not consider the collision between the RBCs and the adhered particles, which may also lead to the dislodgement of particles from the adhered surface. For nonspherical particles, adhesion also depends on the particle orientation, which determines the contact area between the particle and the endothelium and, therefore, the magnitude of the adhesive force, as shown in Fig. 3 (33).

FACTORS AFFECTING MARGINATION

Margination propensity depends on parameters such as the particle size, shape, density, stiffness, and concentration and aggregation state of RBCs. The effects of these

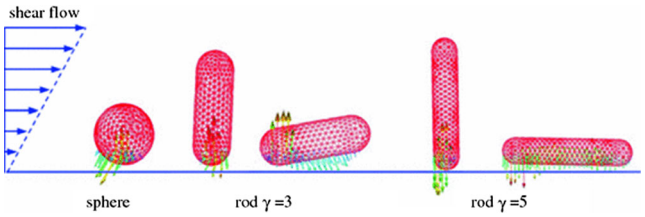

Fig. 3. Shape and orientation dependence of adhesion (33). With kind permission from Springer Science and Business Media

parameters on particle margination are summarized in Table I. However, experimental conditions and assumptions used in the modeling vary considerably, which may explain some of the conflicting results reported.

Particle Size

The size of a particle is an important parameter. Particles larger than 200 nm are at risk of being filtered out of the blood or destroyed by the liver, spleen, and bone marrow, whereas particles less than approximately 10 nm will leave the blood stream through the kidney or via extravasation from a tumor (36). For margination to occur, the particles need to escape the fluid-flow streamlines and move laterally as a result of gravity, buoyancy, hydrodynamic forces, van der Waals forces, and/or Brownian motion (30). In the case of nanoparticles, gravitational forces are usually neglected given the small size of the particles (37). However, as particle size increases, gravitational forces become increasingly important. Gentile *et al.* studied spherical particles with diameters of 50, 100, 200, 500, and 750 nm and 1, 6, and 10 μm (32). They found that gravity facilitated the margination of the larger (>500 nm) particles in the direction of the gravitational force (32). A modeling study by Lee *et al.* confirmed these results and found that for smaller particles (<500 nm), gravity has a negligible effect compared with Brownian motion (38). Toy *et al.* measured the margination of spherical nanoparticles ranging from 60 to 130 nm diameter in a bloodless solution via their adhesion to the walls of a microfluidic channel (30). They observed a faster margination time for smaller particles (Fig. 4a) and attributed it to their higher diffusivity, as explained in "Brownian Motion and Particle Adhesion" (30).

According to a modeling study by Decuzzi *et al.*, gravitational forces dominate far from the endothelium, whereas van der Waals forces dominate close to the endothelium (39). The van der Waals forces cause a "jump into contact" behavior where the particle is suddenly attracted to the endothelium. The authors also found that the competition between electrostatic, van der Waal, steric and buoyancy forces leads to a critical radius where the margination time is the longest, as shown in Fig. 4b (39). Below and above this radius, the nanoparticles marginate more readily (39). The calculated critical radius varies from 50 to 250 nm, depending on a number of factors, such as the particle density, blood ionic concentration, and endothelial cell electrostatic properties (39). It should, however, be noted that the analytical model developed by Decuzzi *et al.* considered the interactions between nanoparticles and endothelial cells only; no interactions between nanoparticles or between blood cells and nanoparticles were included in the model (39). Gentile *et al.* found experimentally, for the discrete bead sizes that they used, that there were two distinct margination mechanisms, based on the particle diameter (32). The 500 nm to 10 μm particles were found to be largely affected by gravity, whereas the 50 to 200 nm particles were found to be largely affected by collodial forces, such as van der Waals forces (32). However, because particles of discrete sizes were used, the exact critical diameter is unknown and the gravitational effect between 200 to 500 nm is unclear.

Charoenphol *et al.* examined the particle size effect experimentally using spheres with a diameter ranging from 0.5 to 10 μm suspended in blood (31). They observed that the margination propensity of particles increased with increasing particle size, as shown in Fig. 4c (31). Namdee *et al.* observed that micron-sized spheres (2 and 5 μm

Table I. Factors reported to affect particle margination

Factor	Observation	Key references
Particle size	Many studies indicate that there is an optimal particle size for margination to occur, but there is no consensus on the exact size. Multiple studies suggested that 500 nm and larger spherical particles exhibited marginating behavior, whereas 200 nm and smaller particles became trapped between RBCs in the core of the blood flow, away from the channel walls	(5,15,30–32,38–40)
Particle shape	Nonspherical particles with a higher aspect ratio have been found to marginate more readily than spherical particles. Particle rotation seems to be a key factor in aiding margination	(5,30,33,38,41)
Particle density	Studies are too different to draw any meaningful conclusions on the general behavior	(5,30)
Particle stiffness	Conflicting simulation results have been reported for WBCs. Freund found that RBC elasticity had no effect on margination of WBCs, whereas Kumar and Graham showed that heterogeneous collisions between a stiff and an elastic particle lead to margination. No studies have been conducted on the sole effect of stiffness on margination	(42–44)
Shear rate	The common use of particle adhesion to quantify the degree of margination leads to inconclusive results about the effect of varying shear rate. Particles may have been detached from the wall due to increasing hydrodynamic drag and/or collision with RBCs	(5,15,30,31,42)
Hematocrit and RBC aggregation	No consensus	(15,26,31,34,42,48,49)

RBC red blood cell, *WBC* white blood cell

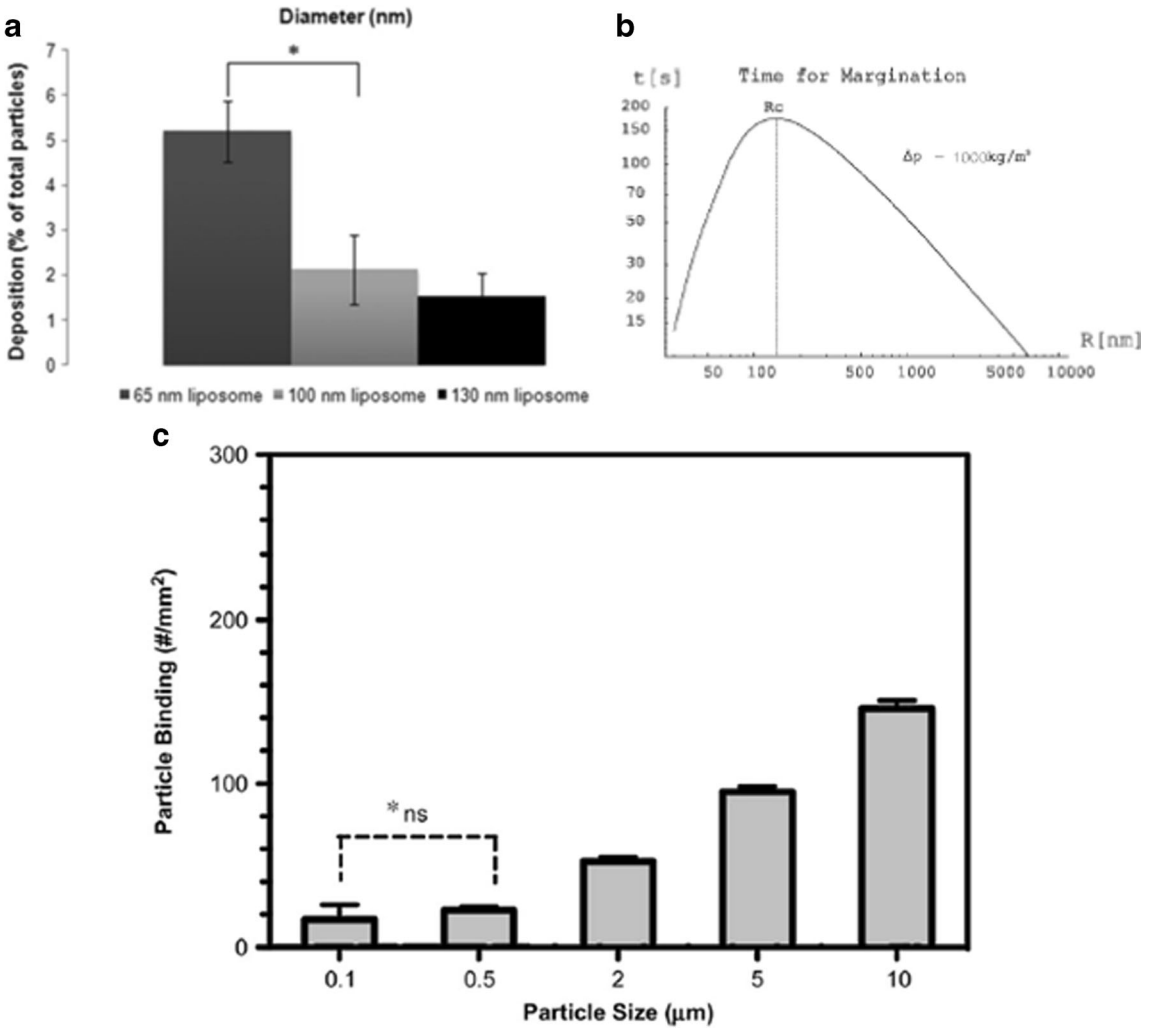

Fig. 4. **a** Liposome adhesion to channel walls for three different liposome sizes (30). Reproduced with permission from IOP Publishing. All rights reserved. **b** Plot of margination time vs. particle size, where R_c is the critical radius (39). With kind permission from Springer Science and Business Media. **c** Particle binding as a function of particle size (31)

diameter) marginated much more effectively than spherical nanoparticles (200 and 500 nm diameter) in a suspension of RBCs (15). They further attributed the reduced margination of nanoparticles to the trapping of nanoparticles within the spaces between the RBCs (15). A similar observation was made by Lee *et al.* in a combined experimental and modeling study (40). In the experimental portion, fluorescent nanoparticles were tracked in the microvasculature of a mouse via intravital video microscopy and 1 μm particles were found to exhibit margination whereas 200 nm particles were found to distribute randomly in the blood vessel, with no apparent tendency to marginate (40). In the modeling part of the study, the model showed that nanoparticles (<100 nm) moved along with the RBCs in the core of flow whereas the larger particles(0.5 to 1 μm) exhibited margination, as shown in Fig. 5 (40).

Particle Shape

Many experiments use only spherical nanoparticles in their study of margination (15,16,31,32,39,40). For nonspherical particles, the physics is more complicated. The velocity gradient in fluid flow results in a nonuniform distribution of forces along the axis of symmetry of the particles, leading to particle rotation. Toy *et al.* studied the margination of gold nanospheres versus that of gold nanorods with a width-to-length ratio of 0.45 suspended in water (30). The gold nanorods were shown to exhibit a much higher margination propensity when compared with the gold nanospheres, as shown in Fig. 6a (30). Likewise, Gentile *et al.* observed that disk-shaped and hemispherical nanoparticles marginated more compared with spherical nanoparticles (5). However, this study focused on the sedimentation of particles and so the differently shaped nanoparticles also had different densities (5). Doshi *et al.* studied how the shape and size of particles influence margination in a bifurcating microfluidic device (41). Particles investigated included spheres of 1, 3, and 6 μm diameter, which were then stretched to form elliptical disks, circular disks, and rod-like particles. Higher aspect ratio particles tended to exhibit greater particle adhesion overall, with the difference becoming more pronounced with larger particle size (41). This could be attributed to the greater contact surface area between the bovine serum albumin (BSA) antibody-conjugated

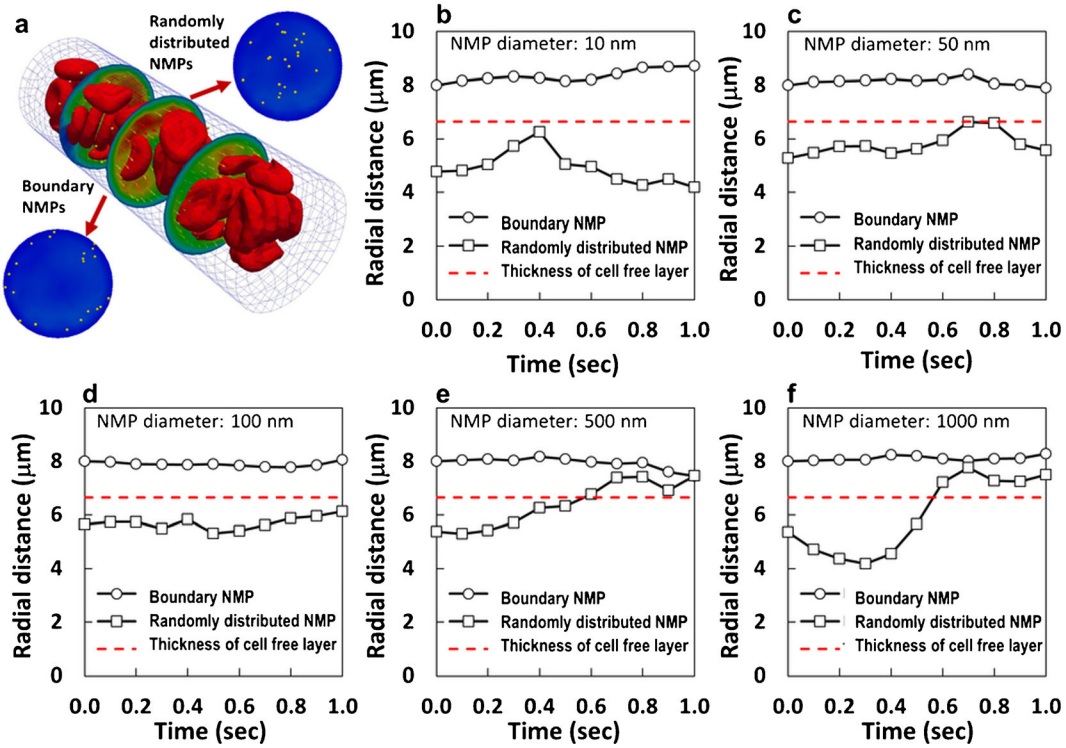

Fig. 5. Model results showing that larger nano/micro particles (NMPs) (500 and 1000 nm) are more likely than smaller particles to cross into the cell-free layer (CFL: represented by the *dashed red line*) and, thus, marginate. Boundary nanoparticles were initially placed in the CFL (40). Reprinted with permission from Macmillan Publishers Ltd: Scientific Reports (40), copyright 2013

particle and the BSA-coated wall, making the higher aspect ratio particles more likely to bind and "stick" to the vessel walls. Lee *et al.* found that higher rotational inertia leads to more pronounced lateral drifting and, thus, a larger margination propensity (38).

For nonspherical particles, margination does not show a clear trend as a function of shear rate. High shear rates can pull the nanoparticles off from the endothelium when particle adhesion is used for the measurement of margination propensity, as previously discussed ("Brownian Motion and Particle Adhesion"). Tan *et al.* simulated spheres with varying particle densities, rods with an aspect ratio of three, and rods with an aspect ratio of five (33). They found that, similar to the results obtained by Gentile *et al.* and Toy *et al.* (5,30), the spheres and the rods exhibited decreasing margination with increasing shear rates (33). They postulated that the trend comes from the competition between the adhesion to the walls and drag forces (33). At low shear rates, the adhesion forces are larger than the drag forces, regardless of the particle orientation. At higher shear rates, these forces depend on the orientation of the particle. If there is only a point contact, then the drag force is stronger than the adhesion force, whereas if a particle's long axis is adhered to the endothelium, then the adhesion force can overcome the drag force, as shown in Fig. 3 (33).

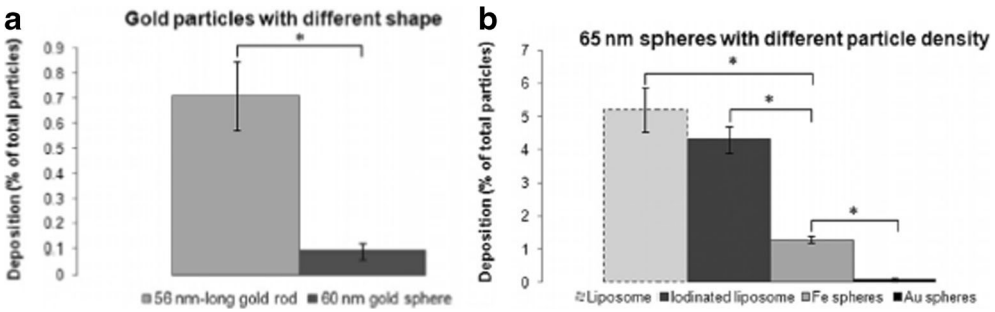

Fig. 6. **a** Adhesion of gold rods with a width-to-length ratio of 0.45 vs. adhesion of gold spheres (30). Reproduced with permission from IOP Publishing. All rights reserved. **b** Adhesion of 65-nm spheres with different densities (30). Reproduced with permission from IOP Publishing. All rights reserved

Particle Density

In an experimental study by Toy *et al.*, 65-nm particles with different densities were studied (30). It was found that nanoparticles with a higher density adhered less than those with a lower density, as shown in Fig. 6b (30). Toy *et al.* explained that the higher-density particles carried more momentum and, as a result, hydrodynamic forces dominated, whereas the low-density particles were dominated by diffusive motion, allowing them to escape streamlines (30). However, when the effects of gravity are included, such as for larger particles, the effect reverses. In Gentile *et al.*'s study, differently shaped particles with sizes of approximately 1 μm had drastically different margination propensities (5). The discoidal particles were 20% heavier and the number of particles marginated was five times larger relative to the spherical particles (5). Likewise, the margination of hemispherical particles that were 60% heavier was three times larger than that of the spherical particles (5). Both Toy *et al.* and Gentile *et al.* studied particles in an aqueous medium with no blood cells. Toy *et al.* used sub-100 nm particles whereas Gentile *et al.* used particles ranging in size from 1 – 3 μm and focused only on their sedimentation to the bottom surface of the flow chamber – not their margination to any wall. This may explain the discrepancy in results between the two studies.

Particle Stiffness

There are two different views on the effect of RBC stiffness on WBC margination. Freund modeled the interactions between RBCs and WBCs and the subsequent effect on WBC margination (42). He increased the stiffness of RBCs by a factor of 10, but the WBCs exhibited only slightly decreased margination (42). The author concluded that the margination of WBCs does not have a strong dependence on the deformability of RBCs and is likely more dependent on the mismatched size or shape of RBCs and WBCs (42). Kumar and Graham mathematically modeled a system comprised of a dilute suspension of neo-Hookean capsules between infinite parallel walls, subjected to simple shear flow (43). Contradicting the results of Freund, they found that stiff particles, such as WBCs, tend to marginate whereas more elastic ("floppy") particles, such as RBCs, undergo the opposite phenomenon and accumulate at or near the center of the channel (43). The study first modeled systems comprised entirely of stiff particles or entirely of elastic particles, and no margination was observed (43). However, upon modeling a system comprised of both types of particles, the stiffer particles were observed to migrate nearer to the wall of the channel and the elastic particles were observed to remain in the center of the channel (43). Kumar and Graham proposed that heterogenous collisions—collisions between stiff and elastic particles—were responsible for the margination of the stiffer particles (43). This accurately describes the phenomenon of RBC aggregation in the center of a blood vessel and WBC margination to the walls of the blood vessels. Interestingly, Kumar and Graham also found that the heterogenous collisions had a much larger displacement effect on the stiffer particles towards the wall than on the elastic particles (43,44).

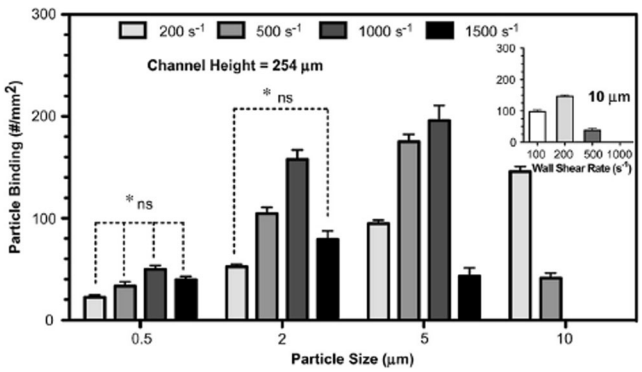

Fig. 7. Adhesion of particles to the wall with varying shear rate (31)

Shear Rate

Shear rate affects the shape of the WBCs or particles, which will in turn modify the adhesion and lift forces acting on those particles. A number of studies showed that WBC margination increases significantly as a function of decreasing shear rate (19,42,45–47). Similar trends have been reported for particles. Toy *et al.* coated the channel walls with fibronectin to capture nanoparticles that marginated and, therefore, came close to the wall (30). They reported that increasing

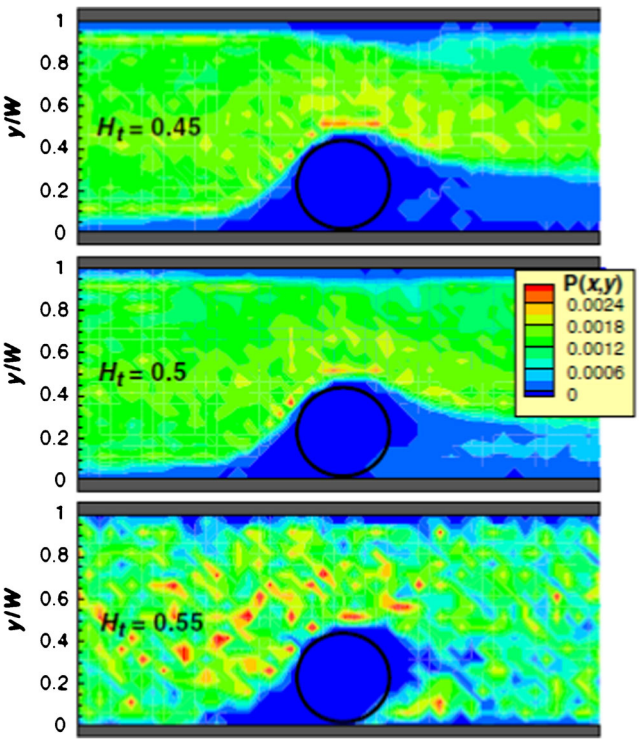

Fig. 8. RBCs interfere with WBC margination at high hematocrits. At a hematocrit of 55%, RBCs move downstream of the marginated WBC and close to the wall. The WBC is traveling faster than these RBCs, and so these RBCs enter the gap between the WBC and the wall and force the WBC away from the wall (26). Reprinted figure with permission from (26). Copyright 2012 by the American Physical Society (http://link.aps.org/doi/10.1103/PhysRevLett.108.028104)

shear rate decreased the margination and, further, attributed this to the detachment of nanoparticles at high shear rates (30). Namdee *et al.* came to a similar conclusion when using 2 and 5 μm spherical particles in an adhesion-based assay (15). Charoenphol *et al.* seeded endothelial cells onto glass and inserted the glass substrate into a flow chamber (31). Particles were bound with ligands, which in turn bound to receptors on the endothelial cells. They observed that particle binding initially increases as a function of shear rate because, for the same particle concentration, the number of particles passing through the channel increases and thus the chance for particles to bind onto the wall increases (31). However, as the shear rate exceeded a certain critical value, the number of particles bound to the wall decreased as a function of shear rate, as shown in Fig. 7 (31). Gentile *et al.* found that the number of particles that marginated via sedimentation has an empirical power–law relationship with the shear rate (5). The exponent varied for differently shaped particles: $\dot{\gamma}^{-0.63}$ for spherical particles, $\dot{\gamma}^{-0.85}$ for discoidal particles, and $\dot{\gamma}^{-1}$ for quasi-hemispherical particles, respectively (5).

Hematocrit and RBC Aggregation

Margination is affected by the interactions between particles and RBCs. Tan *et al.* modeled particle margination with and without RBCs and showed that margination was almost doubled in the case with RBCs (34). The enhanced margination in the presence of RBCs was more pronounced at higher shear rates, where the RBCs tumbled faster. A number of studies investigated the effect of hematocrit—the volume percentage of RBCs in blood—on WBC margination. Fedosov, Fornleitner and Gompper found in their modeling study that the margination of WBCs was highest at hematocrits of 25% and 35% (26). For a hematocrit of 55%, the authors found that RBCs caused a "lift-off" mechanism, wherein the RBCs entered the gap between the WBC and the wall and lifted the WBCs off the wall, as shown in Fig. 8 (26). It should be noted that this "lift-off" mechanism is different from the lift force, as explained in "Inertial, Viscous, and Lift Forces". No explanation was, however, given for why margination of WBCs at 45% hematocrit was lower than that of WBCs at hematocrits of 25% and 35%.

In studies where particle adhesion was used to quantify margination, there is a disagreement over whether hematocrit affects particle margination. Charoenphol *et al.* reported that changes in hematocrit did not affect the margination of spherical particles ranging from 100 nm to 10 μm (31). However, another study by Namdee *et al.* reported that increasing the hematocrit from 30% to 50% increased the adhesion of nanoparticles, but reduced the adhesion of micron-sized particles (15). The decrease in adhesion for the micron-sized particles could be explained by the comparable size of the CFL to the size of the particles, causing RBCs to collide with the particles and knock the particles off of the wall (15). The discrepancy between the Charoenphol and Namdee studies could be a result of the difference in the size of the flow chamber used in each study and thus the thickness of the CFL.

Lastly, there are also conflicting views on whether the aggregation of RBCs affects margination. Jain and Munn perfused a device with dextran solutions and also with a plasma-free culture medium (48). The highest margination propensity of WBCs was observed in the presence of dextran, which induced RBC aggregation at a similar level to that of RBCs in plasma (48). Likewise, Pearson and Lipowsky demonstrated in their *in vivo* experiments that the addition of dextran 500 (Dx500), a high molecular weight dextran known to increase the aggregation of RBCs, increased margination fourfold whereas the addition of dextran 40 (Dx40), a known disaggregating agent, decreased the margination of WBCs by half in the post-capillary venules of rats (49). Furthermore, Fedosov, Fornleitner and Gompper found that the aggregation between RBCs at higher hematrocrits led to a tighter RBC packing and supressed the "lift-off" mechanism (26). However, Freund found in his modeling study that WBCs marginated without RBC aggregation, suggesting that WBC margination does not depend on RBC aggregation (42).

SUMMARY AND OUTLOOK

Margination, initially observed for WBCs, has important implications for cancer diagnosis and therapy. Existing theoretical and experimental studies indicate that margination is influenced by the particle size, shape, density, stiffness, shear

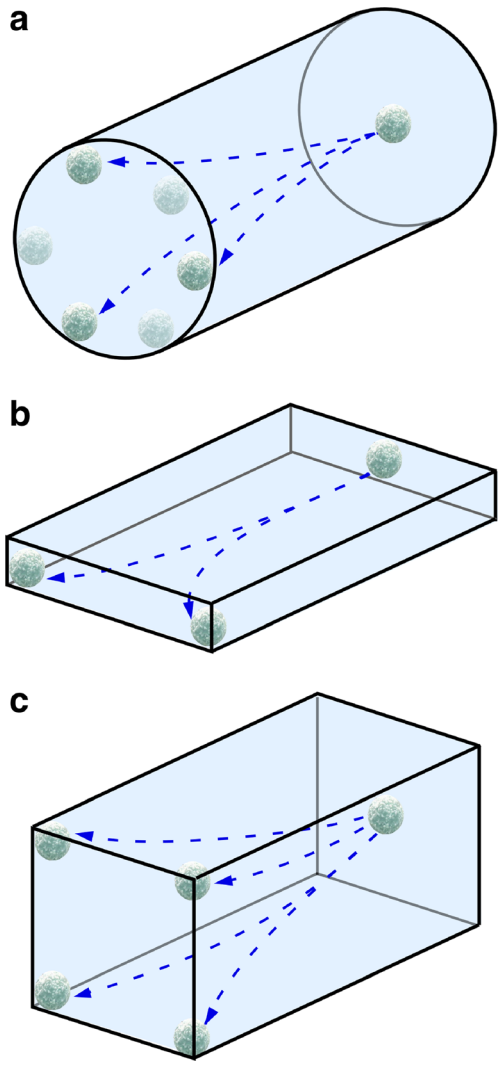

Fig. 9. Margination behavior of WBCs in various channel geometries (50). Reproduced from (50) with permission from The Royal Society of Chemistry (http://dx.doi.org/10.1039/C1LC20293F)

rate, hematocrit, and RBC aggregation, although conflicting results are sometimes reported due to the huge variety in experimental conditions and modeling assumptions. Many studies used particle adhesion in order to quantify margination, but this may further complicate the interpretation of experimental findings. Instead, direct imaging and tracking of particles in microfluidic devices may offer a more reliable way of studying and understanding the fundamentals of margination (14). Recent advances in microfluidics have enabled the design and fabrication of more sophisticated devices, which bear an enhanced resemblance to the actual physiological conditions (50–52). Yang *et al.*, for example, showed that margination is strongly influenced by the geometry of the channels, such as the shape and "sharpness" of a bifurcation (50). They observed that WBCs marginated to the corners of a rectangular channel and that, as a result, it is important for bifurcating devices to have spherical cross-sections in order to mimic the actual arterial network, as shown in Fig. 9 (50). Finally, it is worth noting that most existing studies focus on particles with a relatively simple shape (e.g., cylinders, ellipsoids, disks, and hemispheres). The margination of more complex shapes (e.g., helical) and asymmetric particles with both a stiff and flexible portion remain largely unexplored (53,54). Understanding the flow dynamics and margination of these particles in blood may lead to the rational design of nanoparticles for better diagnostic and therapeutic performance, unlocking the full potential of nanomedicine.

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