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Effect of circulating tumor cell aggregate configuration on hemodynamic transport and wall contact

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Abstract

Selectin-mediated adhesion of circulating tumor cells (CTCs) to the endothelium is a critical step in cancer metastasis, a major factor contributing to the mortality of cancer. The formation of tethers between tumor cells and endothelial selectins initiates cell rolling, which can lead to firm adhesion, extravasation and the formation of secondary metastases. Tumor cells travel through the bloodstream as single cells, or as aggregates known as circulating tumor microemboli (CTM). CTM have increased survivability and metastatic potential relative to CTCs, and the presence of CTM is associated with worse patient prognosis. The motion of cells and cellular aggregates in flow is a function of their size and shape, and these differences influence the frequency and strength of their contact with the endothelium. In this study, a computational model consisting of the hydrodynamic component of the Multiparticle Adhesive Dynamics simulation analyzed the effects of model aggregate conformation and orientation on adhesive binding potential. Model aggregates of the Colo205 colorectal cancer cell line were created, consisting of two, three, and four cells in simple geometrical conformations. Contact time, contact area, and time integral of contact area were measured as a function of fluid shear rate, initial centroid height, and initial orientation for model aggregates that experienced hydrodynamic collisions with the plane wall. It was found that larger CTM conformations with intermediate nonsphericities had the highest adhesion potential. The results of this study shed light on the correlation between environmental conditions and extravasation efficiency, which could inform the development of new anti-metastatic drugs.

Keywords

Cancer metastasis; Circulating tumor cell; Circulating tumor microemboli; Hydrodynamic collision

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Supplementary material

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1. Introduction

Advances in our understanding of the mechanisms underlying cancer growth and metastasis have led to promising developments in cancer detection and treatment methods in recent years [1]. However, despite these advances cancer metastasis remains the cause of approximately 90% of all deaths in cancer patients [2]. The process through which cancer cells extravasate through the endothelium is mediated by highly specific receptor-ligand interactions that allow flowing cells to be brought into contact with and firmly adhere to activated endothelial surfaces before penetrating the subendothelial matrix [3]. The first step of this adhesion cascade is mediated by the selectin family of cell adhesion molecules (CAMs). There are three types of selectins: E-, P-, and L-selectin, which are calcium-dependent trans-membrane glycoproteins that all bind to carbohydrate ligands and share a similar structure. E- and P-selectin are expressed on the surface of endothelial cells stimulated by inflammatory cytokines, and P-selectin is expressed on the surface of activated platelets as well. L-selectin is constitutively expressed on leukocytes, particularly on the tips of microvilli [4]. A common structure recognized by all selectins is the tetrasaccharide sialyl Lewis^x (sLe^x) and its isomer sialyl Lewis^a (sLe^a). sLe^x is the terminal component of glycoproteins and glycolipids found on circulating leukocytes and tumor cells, as well as some endothelial cells [5,6]. Tumor cells such as colorectal cancer cells highly express sLe^x-containing mucin-type glycoproteins that bind to E- and P-selectin, and increased sLe^x expression is correlated with increased metastatic potential and worse patient prognosis [7].

Tumor cells that enter the bloodstream and are able to avoid anoikis, apoptosis due to loss of cell-cell and cell-matrix adhesion, can travel through the bloodstream to distant tissue sites. These circulating tumor cells (CTCs) travel through the bloodstream until they extravasate to distant tissue sites to form secondary tumors [8]. In addition to the presence of CTCs in the bloodstream, circulating tumor microemboli (CTM) have been found in the bloodstream of colorectal, renal, prostate, lung, and breast cancer patients [8,9]. These CTM can result from clusters of cells detaching from the primary tumor and migrating collectively into the bloodstream through leaky blood vessels characteristic of the tumor microenvironment [10]. CTM can also be formed intravascularly; tumor cells attached to the endothelium have been found to recruit other rolling and convecting tumor cells and proliferate. Compared to CTCs, CTM exhibit several survival and prometastatic advantages. CTM are better able to resist anoikis, and the CTM structure may also serve as a protective barrier for the innermost cells from host immunological cells. In addition, CTM benefit from the production of autocrine proinflammatory cytokines and matrix proteases [10,11]. Evidence of the survival and prometastatic advantage of CTM is found in early studies of CTCs and CTM; it has been shown that CTM form metastases more readily than the same amount of single tumor cells injected into the circulation [12]. Despite the relevance of CTM in metastasis formation and growth, research studying its behavior has been limited.

The initial interaction between a circulating tumor cell (CTC) and activated endothelium is a crucial step in the metastatic process that can only occur given the proper balance of forces. The formation of a bond between two molecules requires two major steps: transport, where the molecules are brought into close proximity, and reaction, where the molecules link together. Many biochemical reactions, such as the cytokine activation of endothelial cells,

involve molecules in solution forming complexes after diffusion driven collisions with cell-bound ligands. In contrast, with selectin-mediated binding a moving cell carries adhesion receptors to other cell-bound ligands, thus transport is driven by the relative velocity of involved cells [13]. In steady laminar flow, the velocity of the fluid, and that of flowing particles, increases with the distance from the wall, and this effect is highly dependent on the size and shape of the given particle. At a given distance, larger cells will have higher velocities, as increasing portions of the cell will extend further from the wall. A faster cell velocity means that adhesion molecules will be brought into contact more frequently, but as the velocity increases the contact time is decreased. Thus, optimum binding efficiency depends on the relative timescales of transport and binding reaction [13,14]. This is particularly important with selectin binding.

There has been extensive study of the motion of spherical particles in shear flow, both with and without the presence of a bounding wall, but the study of spherical aggregates is more difficult, as analytical solutions are unavailable for irregular geometries. Given the wealth of theoretical and experimental data on the behavior of spherical particles, studies on nonspherical particles often interpret results relative to theory applicable to spheres. Nonspherical particles can be characterized by commonly used metrics such as aspect ratio and volume-equivalent sphere radius, allowing for meaningful comparisons to be made between particles of different sizes and shapes, and even between different data sets [15,16]. Studies have analyzed the behaviors of nonspherical particles, and those have highlighted the important effects of nonsphericity on transport and hydrodynamic interactions, particularly when in close proximity to a wall [17–22]. These wall-particle interactions alter the trajectories of particles that come in proximity, and such changes can affect the capability of flowing cells to form adhesive interactions. *In vivo*, contact with the endothelium is largely promoted through collisions with blood cells, but *in vitro* assays aimed at understating the behavior of cells in flow utilize dilute suspensions in order to isolate more subtle mechanisms, and in those environments understanding hydrodynamic interactions is vital [23]. Investigating the hydrodynamic behavior of more complicated geometries must be done with numerical models, with inferences drawn from our current understanding of related geometries [24]. The potential for circulating tumor microemboli (CTM) to interact with the endothelium can be investigated using the hydrodynamic component of the Multiparticle Adhesive Dynamics (MAD) simulation.

The Multiparticle Adhesive Dynamics (MAD) simulation is an extension of the adhesive dynamics (AD) simulation originally developed by Hammer and Apte as a numerical simulation of a rigid sphere with randomly distributed surface ligands interacting with a receptor-coated plane surface under viscous shear flow. The AD simulation can recreate the wide range of adhesive phenomena displayed by leukocytes in postcapillary venules [25]. The MAD simulation, created by King and Hammer, was an adaptation of the original AD model that included multiple particles to observe particle–particle interactions in addition to cell adhesion to surfaces [26]. The MAD simulation consists of two interconnected components: a hydrodynamic calculation of the mobility of a rigid sphere in low Reynolds shear flow computed using the Completed Double Layer-Boundary Integral Equation Method (CDL-BIEM) and a Monte Carlo based adhesive dynamics simulation of receptor–ligand bond dissociation assuming Bell model or catch-slip bond reaction kinetics

[23,26,27]. The method used by the MAD simulation to calculate rigid body motion, the Completed Double Layer-Boundary Integral Equation Method (CDL-BIEM), is one that can be applied to any arbitrarily shaped particle as long as its surface is continuous and relatively smooth, and it is well suited to model circulating tumor microemboli [28,29]. The use of a model system with linear shear flow and a flat plane serves as a representation of the flow conditions in linear portions of blood vessels, where the estimated range of blood flow forces are shear rates between $150\text{--}1600\text{ s}^{-1}$, and resultant shear stresses between 0.8 and 4 dyne/cm², depending on the vessel size and blood flow rate [30,31]. Prior numerical models successfully replicated the formation and maintenance of CTC aggregates in flow [32–36], but they are lacking in information on the interaction of aggregates with the endothelium. This novel adaptation of the MAD simulation, consisting of model colorectal cancer cell line aggregates in linear shear flow in the presence of a single plane wall coated with E-selectin, serves as a simplified model that describes the behavior of CTM as they make initial contact with activated endothelium. A schematic of the MAD simulation is displayed in Fig. 1.

In this study, we carried out simulations to analyze the hydrodynamic behavior of model CTM at various heights, shear rates, and orientations near a plane wall, to observe the trajectories of CTM and estimate their potential for adhesive contact. We used observations made from the Colo205 colorectal cancer cell line as an example for physical mechanisms of wall interaction. To adapt the model to describe Colo205/E-selectin interactions, relevant parameters were obtained for the MAD simulation. The hydrodynamic behavior was validated against analytical solutions for spherical aggregates in low Reynolds number flow. Model CTM geometries were then systematically tested under physiologically relevant flow conditions to observe hydrodynamic collision events between model aggregates and the plane surface.

2. Methods

2.1. Cell culture

The human colorectal adenocarcinoma cell line Colo205 (ATCC number CCL-222) was obtained from American Type Culture Collection (Manassas, VA) and maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma) with 10% (v/v) fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin at 37 °C in a 5% CO₂ incubator. Cells were imaged using an Olympus IX81 inverted microscope (Olympus America Inc., Melville, NY), linked to a monitor and Sony DVD Recorder DVO-1000MD (Sony Electronics Inc., San Diego, California).

2.2. Electron microscopy of cell surface

Colo205 cells were released from the culture flask using Accutase (MP Biomedicals, Solon, OH), washed once with phosphate-buffered saline (PBS) (Invitrogen, Camarillo, CA), and placed on ice. Cells were fixed for 2 h in 2% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA) on ice. Cells were subsequently treated with 1% osmium tetroxide (Electron Microscopy Sciences) for 1 h on ice. Fixed cells were dehydrated with successive 5 min washes of 25% and 50% ethanol. Cells were allowed to sit in 75% ethanol overnight at 4 °C and then washed twice for 5 min in 100% ethanol. Cells were subjected to critical point

drying with carbon dioxide, mounted on metal stubs with copper tape, and coated with carbon. These instruments were provided by the Cornell Center for Materials Research. Samples were stored in argon at room temperature and then imaged with a Zeiss Ultra scanning electron microscope (SEM) at magnifications of $\times 6000$ to $\times 30,000$ in the Cornell Nanofabrication Facility.

2.3. Quantification of Colo205 morphology

Optical light and scanning electron microscopy images of cultured Colo205 cells and cell clusters were analyzed using ImageJ, an open source image processing software available at [<https://imagej.nih.gov/ij/index.html>]. For the optical microscope images, the major and minor axes of 85 cells were measured using the straight line tool, and those values were averaged to determine mean cell diameter. The cell surface roughness layer was determined through ImageJ analysis of the electron microscopy images. In this model cells are assumed spherical, so surface roughness elements were defined as bumpy features that extended beyond the spherical shape of the cell. In ImageJ the spherical boundary was traced, and then paired points were recorded from the boundary of the cell to the height of surface features to determine their heights. A total of 181 surface roughness elements were measured, and the surface roughness layer length was defined as the mean surface roughness feature height. Averaged values were reported as mean $\pm$ SD.

2.4. Generation of Multiparticle Adhesive Dynamics simulation model particles

An algorithm was developed using MATLAB software (The MathWorks Inc., Natick, MA) to modify the original sphere geometry used in the MAD simulation. Model particles in the original iteration of the MAD simulation were spheres discretized into QUAD9 elements, quadrilateral elements with three nodes per side and one central node, for a total of 9 nodes per element. The 96-element spherical mesh was generated by starting with a cube, dividing each cube face into 16 quadrilateral elements, and then projecting the cube onto the surface of a sphere placed at the cube center [26,37]. Mesh geometries were developed using the 96 QUAD9 element sphere as a template, and modified using morphological data collected from Colo205 cell aggregate samples. Mesh geometries were created for 2–4 cell aggregates in idealized conformations of those observed in *in vitro* studies.

To create a doublet mesh, the developed algorithm removed elements from one face of the sphere and then created a duplicate of it. The duplicated sphere was then reoriented so that the opened faces were aligned towards each other and the two spheres were in contact with each other. The nodes along the open face of each sphere were then merged so that the mesh consisted of one continuous set of elements. A diagram of this process is given in Fig. 2. This algorithm allowed any number of faces to be removed from the original sphere, and a corresponding sphere duplicate attached to that side. Attachments were also made to any duplicated spheres in the aggregate, rather than the original. The coordinates for each monomer were adjusted in tandem or separately to create CTM that match the morphology of imaged Colo205 cells. For the experiments, idealized aggregates were used. Mesh input files were generated for doublet, triplet, and 4-mer aggregates of equally sized spheres, with multiple conformations for triplet and 4-mer aggregates.

2.5. Hydrodynamic calculation

Hydrodynamic calculation using the CDL-BIEM is described in detail by Kim and Karilla [38], and a brief overview is given here. Physiological shear flow in postcapillary venules (0.8–4 dynes/cm² shear stress), is well within the Stokes flow regime [25,30], so the Stokes approximation of the Navier-Stokes equations can be used to describe the motion of the fluid,

$$-\nabla p + \mu \nabla^2 \mathbf{u} = 0, \quad \nabla \cdot \mathbf{u} = 0 \quad (1)$$

where p is the pressure, μ is the fluid viscosity, and $\mathbf{u}$ is the velocity. Flow is assumed incompressible. The ambient flow is taken to be linear shear flow,

$$\mathbf{u}_\infty = Gz \quad (2)$$

where G is the shear rate and z is the distance from the wall. A no-slip condition is given at the surface of the wall, so the velocity is zero.

$$\mathbf{u}_0 = 0 \quad (3)$$

No-slip conditions are also given at the surface of each of the N particles,

$$\mathbf{u} = \mathbf{U}_\alpha + \boldsymbol{\omega}_\alpha \times (\mathbf{x} - \mathbf{x}_\alpha) \quad \mathbf{x} \in S_\alpha \quad (4)$$

where $\mathbf{U}_\alpha$ and $\boldsymbol{\omega}_\alpha$ are the translational and rotational velocities of particle α , respectively, and $\mathbf{x}_\alpha$ and S_α are the center of mass and surface of particle α . The motion of an isolated cell is related to the forces acting on it by the 6×6 mobility matrix $\mathbf{M}$:

$$\mathbf{u} = \mathbf{M}\mathbf{f} \quad (5)$$

where $\mathbf{u}$ is a six-element vector containing the particle's translational and rotational velocities, and $\mathbf{f}$ is a six-element vector containing the three components of net force and torque acting on the particle. The expanded mobility matrix relation is given in Eq. (6):

$$\begin{pmatrix} V_x \\ V_y \\ V_z \\ \Omega_x \\ \Omega_y \\ \Omega_z \end{pmatrix} = \begin{pmatrix} \mathbf{M}_{VF} & \mathbf{M}_{\Omega F} \\ \mathbf{M}_{VT} & \mathbf{M}_{\Omega T} \end{pmatrix} \begin{pmatrix} F_x \\ F_y \\ F_z \\ T_x \\ T_y \\ T_z \end{pmatrix} \quad (6)$$

where V_x , V_y , and V_z are the Cartesian components of linear velocity, Ω_x , Ω_y , and Ω_z are the Cartesian components of angular velocity, F_x , F_y , and F_z are the Cartesian components of force, and T_x , T_y , and T_z are the Cartesian components of torque.

The mobility matrix relates the components of force and torque with the components of linear and rotational velocity, as shown by the 3×3 submatrices $\mathbf{M}_{VF}$, $\mathbf{M}_{\Omega F}$, $\mathbf{M}_{VT}$, and $\mathbf{M}_{\Omega T}$. For an isolated sphere near a plane wall in Stokes flow, all of the components of the mobility matrix are known [20,39,40], so if the net force and torque components acting on the particle are summed the instantaneous particle velocities can be determined from Eq. (5). The integral representation of the Stokes equation is

$$u_j(X) + \int_S H_{ij}(\mathbf{x}, X) u_i(\mathbf{x}) dS(\mathbf{x}) = \int_S G_{ij}(\mathbf{x}, X) t_i(\mathbf{x}) dS(\mathbf{x}) \quad (7)$$

where $H_{ij}(\mathbf{x}, X)$ is the i th component of the stress tensor of G_{ij} , G_{ij} is the singularity solution resulting from a point force in the vicinity of a plane at $\mathbf{x}$ in the j th direction, $t_i(\mathbf{x}) = \sigma_{ki} n_k$ is the stress tensor at $\mathbf{x}$, and $\mathbf{n}$ is the outwardly directed unit normal on S . The integral on the right side of the equation is called the single-layer potential, and the integral on the left hand side of the equation is called the double-layer potential. Eq. (7) is a boundary integral equation (BIE) when X is evaluated at the particle surface ($X \in S$). A BIE containing only the single layer potential is sufficient to represent the Stokes flow problem for rigid body motion of particles, but this equation takes the form of a Fredholm integral equation of the first kind, with is generally ill-conditioned for a mobility problem. To circumvent this issue, the BIE containing the double layer potential is used. However, the double layer potential in itself is incapable of exerting any force or torque on the fluid, resulting in an indeterminate system containing null solutions. To complete the system, the forces and torques acting at the center of each particle are coupled with the null solutions on the particle surface to produce $6N$ additional linearly independent equations needed to make the double layer BIE fully determinate. The rigid body motions of each particle can then be extracted from the solution of the BIE and used to update the nodal points on each particle surface, centroid of each particle, and bond endpoints [21,26].

Both the particle and the plane surface were coated with a steric layer to model the natural roughness of the cell glycocalyx layer. This surface roughness layer follows the assumption that both surfaces are covered with a bumpy region dense enough to cover the cell, but dilute

enough for fluid to pass through. As a result this surface roughness layer is considered to have a negligible impact on the hydrodynamic calculations. The adhesive and repulsive interactions are exerted on the tips of these roughness elements [21,26].

A very short-range, general repulsive force equation was included in the model to account for nonspecific interactions such as electrostatic repulsion:

$$F_{rep} = F_o \frac{\tau e^{-\tau \epsilon}}{1 - \tau e^{-\tau \epsilon}} \quad (8)$$

where F_o is the magnitude of the repulsive force, $1/\tau$ is an interaction length scale on the order of angstroms, and ϵ is the separation distance between the two surface roughness layers. The repulsive force is directed normal to the plane wall. F_o and τ are experimentally determined to provide a force strong enough to prevent particle-wall overlap while allowing the surfaces to come within reactive distances of each other [21,26]. An exponential relation of similar form was used by Bell et al., and this general form is used in Stokesian dynamics simulations [18,41].

2.6. Numerical implementation

The Multiparticle Adhesive Dynamics model was modified from the configuration optimized for leukocyte/P-selectin interactions to describe the interaction between Colo205 cells and E-selectin. The MAD simulation code was written in FORTRAN 95 and compiled with the GNU Fortran Compiler; double precision was used for all calculations. A dynamic timestep was incorporated into all simulations; the timestep was reduced as the number of iterations required for convergence increased, which typically coincided with decreased cell-to-surface distance. The maximum timestep was set to 10^{-7} s. Eq. (8) was employed for the repulsive force calculations, with experimentally determined values for the magnitude of repulsive force F_o and repulsive force interaction range τ . Information obtained through new *in vitro* measurements and the literature were used to define the values for the remaining parameters.

2.7. Hydrodynamic validation of the model

To validate the hydrodynamic behavior of the CTM, particles were placed at an initial centroid height 30 volume-equivalent sphere radii from the wall, with major axes oriented perpendicular to flow, along the x -axis, and any cell protrusions oriented in the positive y - and z -axis direction. At this distance, wall effects could be safely neglected [19]. The shear rate was set to 1000 s^{-1} , and the CTM were allowed to translate and rotate in fluid flow for a simulated time of 1 s. For the doublet, the rotation rate Ω vs. orientation angle θ was compared to the general expression given by Nir and Acrivos [42]

$$\Omega = \frac{d\theta}{dt} = \frac{1}{2} G (1 + C \cos 2\theta) \quad (9)$$

where G is the shear rate, and C is a constant defined as

$$C = \frac{r_e^2 - 1}{r_e^2 + 1} \quad (10)$$

Here, r_e is the ellipsoid equivalent axis ratio. The equivalent axis ratio for a rod-like chain of spheres was determined using the semi-empirical relation given by Harris and Pitman [43]:

$$r_e = 1.14a_r^{0.844} \quad (11)$$

where a_r is the true aspect ratio of any axisymmetrical particle, defined as major axis length/minor axis length. The aspect ratio of the doublet was 1.929, resulting in a C value of 0.576. For the triplet and 4-mer rods, the rotation rate vs. orientation angle was compared to Jeffery's equation for the rotation rate of an ellipsoid in unbounded shear flow [44]:

$$\frac{d\theta}{dt} = \frac{G}{r_e^2 + 1} (r_e^2 \sin^2 \theta + \cos^2 \theta) \quad (12)$$

The aspect ratios for the triplet and 4-mer were 2.859 and 3.788, respectively. All rotation rates were normalized by the shear flow rate. The normalized root-mean-square deviation (NRMSD) was calculated for each rotation rate to determine the model error. The root-mean-square deviation (RMSD) was defined as

$$\text{RMSD} = \sqrt{\frac{\sum_{i=1}^n (x_i' - x_i)^2}{n}} \quad (13)$$

where n is the number of data points, x_i is the observed value at i , and x_i' is the theoretical value at i . The RMSD was normalized by

$$\text{NRMSD} = \frac{\text{RMSD}}{x_{i, \max} - x_{i, \min}} \quad (14)$$

The Stokes drag force for aggregate conformations was compared with available analytical solutions. The drag force on a sphere in low Reynolds shear flow is given by

$$F_D = 3\pi\mu Ud \quad (15)$$

where μ is the fluid viscosity, U is the fluid velocity relative to the sphere, and d is the sphere diameter. For non-spherical particles, the drag force must be modified:

$$F_D = 3\pi\mu U d_e K \quad (16)$$

The sphere diameter must be replaced with the volume-equivalent sphere diameter d_e

$$d_e = \left(\frac{6}{\pi} \text{Volume} \right)^{1/3} \quad (17)$$

and a shape factor K must be added. As an object's nonsphericity increases, the surrounding flow field becomes increasingly dissimilar to that of a sphere, causing the drag to be higher than predicted using the standard Stokes flow equation [45]. Shape factor values for some spherical aggregates have been empirically determined through flow experiments, and shape factor values for aggregates used in this study are given in Table 1 [16,46]. The relative error was also determined for the drag force calculations.

2.8. Evaluation of model aggregate collision events

A series of simulations were run with model CTM geometries placed with an initial centroid height of 0.25 volume-equivalent sphere radius R_e from the surface, with major axes oriented parallel to flow, with minor axes centered at the origin, and allowed to translate and rotate freely at shear rates of 500, 1000, and 2000 s^{-1} for a simulated time of 1 s. As the hydrodynamic component of the MAD simulation is deterministic, no duplicate simulations were necessary. A second series of simulations was run with model particles placed at an initial centroid height of 0.25, 0.5, 0.75 and 1 R_e with arbitrary orientations, and allowed to translate and rotate freely at a shear rate of 1000 s^{-1} .

A model particle was considered to have undergone a collision event if any node of the particle came within a reactive binding distance with the plane surface. Reactive binding distance was defined as surface roughness layers being at a distance less than or equal to the equilibrium length of an E-selectin/sLe^x bond, 20 nm. Collision contact time and contact area were recorded for each collision, and time integral of contact area was recorded for each model CTM particle. Time integral of contact area (TICA) is a measure of relative adhesion probability [21], defined as

$$\text{TICA} = \int_{t_i}^{t_i + t_c} A_c dt \quad (18)$$

where t_i is the initial time of collision, t_c is the contact time during collision, and A_c is the contact area, or the surface area of the particle within reactive distance to the plane wall. Simulation times were normalized by the shear rate, and distances were normalized by the volume-equivalent sphere radius R_e . Table 2 lists the R_e values for each model CTM. In addition to recording the contact time, contact area, and TICA for each collision, the simulation recorded instantaneous position, orientation angle, and time every 100 time steps.

MATLAB algorithms were developed to visualize the simulations. One algorithm was created to produce a video of the trajectory of the particle over time, with each video frame containing multiple views of the particle simultaneously, as well as a dynamic plot of instantaneous velocity. Another MATLAB routine was created to produce a collision map of model particles, with particle nodes colored based on contact time. The minimum color map value was set equal to the minimum nonzero node contact time, and the maximum color map value was set equal to the maximum node contact time. For the simulations where particles were placed at varying initial centroid heights, the contact time plotted for a given node was the sum of contact durations from each particle.

3. Results

3.1. Establishment of model CTM geometries

To demonstrate the capability of the mesh generation algorithm to create relevant model particles, doublet, triplet, and 4-mer particle meshes were created to match the morphology of imaged Colo205 CTM, shown in Fig. 3. Accurate representations of imaged CTM could be made by increasing or decreasing monomers relative to the average size, and adjusting the coordinates at the intersections between monomers.

For subsequent experiments, CTM displayed in Fig. 4 were used; all aggregate monomers had a radius of $6.959\text{ }\mu\text{m}$, and connections between monomers occurred through the central 4 QUAD9 elements of each sphere face. A 184 element doublet mesh was created for the conformation of two equally sized cells in contact. For the triplet, two conformations were created. A 272 element triplet arranged as a rod-like chain of three cells, and a 272 element triplet in a triangular formation. For the 4-mer, three conformations were created: a 360 element rod-like chain of spheres, a 352 element square, and a 360 element tetrahedron.

3.2. Simulation parameters

Table 3 lists the input parameters for the MAD simulation. The mean cell diameter measured for Colo205 cells was $13.92 \pm 2.42\text{ }\mu\text{m}$, and the mean surface roughness feature height was $270.6 \pm 148.1\text{ nm}$. Fig. 5 displays examples of the optical and SEM images used to quantify Colo205 morphology. For parameter values obtained from the literature, the relevant study is cited.

3.3. Validation of simulation behavior

The rotational rate of rod-like doublet, triplet, and 4-mer aggregates placed far from the plane surface was compared to theoretical solutions for the rotation of rod-like particles in unbounded shear flow. Fig. 6 shows that the results were in excellent agreement. The relative error for the rod-like doublet, triplet, and 4-mer was 2.68, 0.88, and 1.63%, respectively. At $\theta = 0$, the particles were oriented with their major axis parallel to flow, and as can be seen from the graph, the largest angular velocity occurred when the particles were oriented perpendicular to flow. The particles exhibited very similar periodicities of rotation, and the small difference from theory can be attributed to discretization error.

The Stokes drag force of model aggregates placed far from the plane surface was compared to theoretical solutions for the Stokes drag force of simple sphere clusters, shown in Fig. 7. These results were also in excellent agreement; the relative errors were 0.006, 6.3×10^{-4} , 0.26, 0.001, and 0.009% for the doublet, triplet-triangle, triplet-rod, 4-mer-square, and 4-mer-rod, respectively.

3.4. Effect of circulating tumor microemboli conformation and shear rate on adhesion potential

Trajectories were compared for model CTM particles oriented parallel to flow. A representative video of a particle trajectory, shown for a triplet-triangle, is included as a supplemental file (Movie S1). For a given model geometry, the centroid of the particle oscillates toward and away from the plane wall, in a trajectory that maintains a repeating pattern dependent on the mesh configuration and initial particle orientation. The lowest point of the particle was monitored, and a collision event was marked by a blunting of the oscillation pattern. An example of this is highlighted in Fig. 8, a trajectory for a 4-mer tetrahedron. In the absence of a wall interaction, the particle low point naturally oscillated as a result of the particle rotating, smoothly decreasing in height as the aggregate monomers moved towards the surface and increasing in height as the monomers moved away. During a collision, the particle would continue to translate and rotate, but being in contact with the surface, the low point could not descend any lower. As a result, during the contact the low point height was found to plateau at a minimum, until the particle moved away from the surface and the low point height increased and resumed a regular pattern.

Particle trajectory was unaffected by changes in shear rate. The oscillation pattern for a given particle geometry at each shear rate was the same, with only minor differences in the local minima and maxima of centroid and low point heights. However, as the shear rate increased, the wavelength of the pattern decreased. For a given particle geometry, normalizing the simulation time by the shear rate and plotting the three trajectories on the same figure resulted in the low point curves overlapping, as seen in Fig. 8.

Hydrodynamic behavior for all simulations was stable. The simulations persisted over an extended period of time, and in the absence of any collision interactions the model particles oscillated in a repeating pattern indefinitely, with the same approximate maximum and minimum trajectory height. This suggests that the interaction range of repulsive force chosen for the simulation was sufficiently short range. An example of an extended trajectory can be seen in Fig. 9, shown for a model doublet.

All of the horizontally oriented particles made contact with the wall, then exhibited behavior known as “pole vaulting.” Trajectory behavior was similar for all horizontally oriented particles, but a representative trajectory, shown for a triangular triplet, is displayed in Fig. 10. As seen in Fig. 10A, fluid flow induced particle translation and rotation, which periodically brought aggregate monomers in proximity with the plane surface. Particle velocity was proportional to centroid height, and reached a minimum when the particle made contact with the plane surface (Fig. 10C). The centroid height also reached a minimum during the collision, and after colliding the particle was driven from the surface and reached a centroid height higher than its initial position (Fig. 10B). This change in centroid height as

a result of a collision is referred to as a “pole vault” event. After forming contact with the surface, the horizontally oriented particles all adopted new repeating trajectories at centroid heights higher than their initial positions, and they did not return to a reactive distance with the surface.

Due to the orientation of the particles and the flow field, rotational motion for the rod-like and 4-mer-square aggregates occurred only about the y-axis, and points of contact were limited. Collision maps showing the cumulative node contact duration for each particle geometry are shown in Fig. 11. These maps correlated the cumulative frequency of contact for particles at all three observed shear rates to color intensity. Upon forming contact, the rotation of the rod-like particles decreased and contacts formed along the centerline of the particles. Particles with greater aspect ratios experienced a greater decrease in rotation upon collision, as seen by the higher maximum contact for the triplet rod vs. the doublet rod (Fig. 11A–D). Pole vaulting altered the particle trajectories such that minimal contact occurred on the trailing edge, and for the 4-mer square aggregate the contacts only occurred on the side of the aggregate that formed the initial collision (Fig. 11E). The 4-mer square aggregate was also the only geometry configuration to have contact occur on two monomers simultaneously.

Total contact time, maximum contact area, and time integral of contact area were quantified and compared for the different particle conformations (Fig. 12). As expected, the contact time for a given aggregate conformation decreased with increasing shear rate, though the maximum contact surface area remained constant (Fig. 12A and B). The independence of maximum contact surface area on shear rate is consistent with particle trajectories being independent of shear rate. The triangular triplet had the highest total contact time, followed by the rod-like and square 4-mers. The doublet had the lowest total contact time (Fig. 12A). The maximum contact surface area was similar for the triangular and rod-like triplets, rod-like 4-mer, and 4-mer tetrahedron. The maximum contact area was lowest for the doublet, and the 4-mer square had the highest maximum contact area by far (Fig. 12B). The time integral of contact area (TICA), which effectively combines contributions of collision duration with instantaneous contact area, decreased with increasing shear rate proportionately to the decrease in total contact time (Fig. 12C). The model doublet CTM had the lowest contact time, contact area, and TICA of any aggregate conformation. Both triplet conformations and the 4-mer rod and tetrahedron conformations had similar maximum contact areas, but the total contact time for the triplet conformation was about twice as long as for other conformations with similar maximum contact areas. As a result, the triangular triplet conformation displayed the highest time integral of contact area. The 4-mer square configuration had a maximum contact surface area over five times as large as any other conformation, but it had the third highest total contact time, and third highest TICA.

3.5. Effect of circulating tumor microemboli conformation and initial centroid height on adhesion potential

Trajectories were compared for the arbitrarily oriented model CTM particles set at a range of initial heights. For each mesh geometry, simulations were run for 3 randomly generated orientations per initial height. Fig. 13 displays which combinations of initial height and

model particle geometry were able to form contacts with the plane wall. For combinations marked with an “X,” at least one simulation in the set was capable of forming contact with the surface. Collisions only occurred for model CTM with initial centroid heights less than or equal to $0.5 R_e$, and no collisions were observed for any doublet or 4-mer rod-like aggregates. When collisions did occur, pole vaulting was again observed, and particle trajectories that began at both $0.25 R_e$ and $0.5 R_e$ stabilized at around the same height. After stabilizing at the new height, model aggregates did not form collisions again. This behavior was observed for all arbitrarily oriented geometries that formed a contact; a representative illustration of this effect of initial heights on particle trajectories and collisions, shown for a rod-like triplet, is displayed in Fig. 14.

For model geometries where collisions were observed, the total contact time, maximum contact area, and time integral of contact area (TICA) recorded for each initial orientation were averaged, and those averaged values were used to compare collision metrics between geometries. Averaged values were plotted as mean $\pm$ standard deviation. No collisions were observed for any doublet or rod-like 4-mer particles, so no collision metrics were obtained for these cases. For the particles that were able to undergo collisions, similar contact time, maximum contact area, and time integral of contact area were observed (Fig. 15). The mean total contact times observed in arbitrarily oriented particles was also similar to the contact times observed for horizontally oriented particles. Total contact times for arbitrarily oriented particles ranged from 1.9 to 3.0 ms (Fig. 15A), and total contact times for those same particles, when initially oriented horizontally, ranged from 1.7 to 4.2 ms (Fig. 15A). Maximum contact areas for horizontally and arbitrarily oriented particles were similar for triplet conformations as well. For the square 4-mer, however, the contact areas differed greatly. The maximum contact area for the square 4-mer decreased from $17.574 \mu\text{m}^2$ (Fig. 15B) to an average of $3.284 \pm 0.319 \mu\text{m}^2$, similar to the maximum contact areas observed for the other conformations (Fig. 15B). Fig. 15C displays the averaged TICA for the randomly oriented particles, which serves as a measure of relative adhesion potential. Though the rod-like triplet aggregate had the highest mean value, given the standard deviations the differences do not reach statistical significance. Averaging the TICA values of the randomly oriented aggregates minimized the differences in adhesion potential between aggregate conformations seen when the aggregates were all horizontally oriented.

4. Discussion

The MAD simulation displayed excellent stability; in the absence of interaction forces model aggregates adopted trajectories that remained at consistent heights and continued indefinitely. Model aggregate trajectories oscillated in repeating patterns dependent on their initial height and orientation. This repeating oscillatory pattern, dependent on particle geometry, initial height, and initial orientation, is similar to the phenomenon characterized by Jeffery [44]. Pole vaulting behavior has been previously observed for numerical and experimental studies of rod-like particles, and the magnitude and frequency of this behavior is a function of nonsphericity [18,47,48]. As seen in Fig. 14, particle trajectories stabilized at a height independent of their initial centroid height. This is a result of the repulsive force, which is inversely proportional to distance. During contact, the distance reached a minimum, and as a result the same amount of repelling force was applied to the particle. The

consistency of simulation results with prior studies suggests that appropriate values for repulsive force parameters were chosen.

As expected, when trajectory curves were plotted for a given model aggregate conformation in flow, set at the same initial height but induced by different shear rates, the curves overlapped one another once simulated time was normalized by fluid shear rate (Fig. 8). This can be attributed to the linearity of the Stokes equations; streamlines and fluid motion are independent of the magnitude of shear force. Also, in the absence of external forces such as gravity, wall-particle interactions are reversible. However, as seen in Fig. 8 the three trajectories of a 4-mer tetrahedron did not perfectly overlap after nondimensionalization of simulated time. There were slight differences in the local maxima and minima of the oscillation pattern. This was due to the inclusion of the repulsive force interaction, which is not a function of shear rate.

When the model CTM particles were oriented parallel to the wall, every aggregate conformation tested formed contact with the plane wall. Due to the symmetrical orientation of the particles about the axes, rotation for the rod-like and square aggregates only occurred about the y-axis, and there were minimal points of contacts (Fig. 11). For the majority of conformations, the maximum contact area was relatively similar, but due to the shape of the square 4-mer aggregate, its maximum contact area was over 5 times that of the other conformations when the particles were initially oriented horizontally (Fig. 12B). Despite the square 4-mer having a much higher maximum contact area, the triangular triplet displayed the highest contact time and time integral of contact area. This may be due to the relatively intermediate sphericity of the triangular triplet. The square 4-mer and other more nonspherical geometries such as the rod-like particles, spent more time oriented parallel to flow, where any point of the particle would be sufficiently far from the wall to avoid collisions. The collision map in Fig. 11E and F shows that contact only occurred on one side of the particle, whereas it can be seen from the triangular triplet's trajectory in Fig. 10A that the triplet rotated in such a way that regardless of its orientation, a protrusion typically remained near the wall.

When the particles were given arbitrary orientations and varying initial heights, no collisions were formed for the CTM placed at $0.75 R_e$ or $1 R_e$ from the surface. This behavior complemented that seen in other studies of particles in the presence of a wall [22,37]. Unlike the other geometries, doublet and rod-like 4-mer particles did not form collisions at any initial height. Due to constraints on computational resources, only a limited number of orientations could be run, and as a result the orientation angles represented by the experimental groups did not necessarily have a varied distribution. If enough initial orientations were simulated, it is likely that collisions would be seen for all conformations at initial centroid heights of $0.25 R_e$ and $0.5 R_e$. As observed with the particles with fixed orientations, these geometries were capable of forming contacts with the plane surface given the proper initial orientation. In linear shear flow, the presence of a wall induces a lift force on nonspherical particles. This force is at a maximum at an orientation angle $\theta = \pi/4$, and the effect is most pronounced on particles with axis ratios ≈ 2.85 [19]. This susceptibility to lift forces highlights a potentially important distinction in comparing the binding potential of cellular aggregates. The potentially increased dependence of doublet and rod-like 4-mer

particles on an ideal initial orientation to form contacts implies that these conformations may have lower binding potential than the other conformations. The time integral of contact area is a useful metric for estimating relative binding potential, as it effectively combines the contributions of collision duration with instantaneous contact area. For the randomly oriented aggregates that did form geometries, the relative binding potentials, as well as the total contact time and maximum contact area, were similar. This contrasts with the horizontally oriented particles, which showed clear differences in binding potential. Interestingly, the 4-mer square particle had a greatly decreased max contact area, but a similar binding potential, suggesting that contact time may contribute more to binding potential than the surface area.

5. Conclusions

The hydrodynamic component of the Multiparticle Adhesive Dynamics simulation successfully recapitulates the behavior of cells interacting with a plane wall, and it allows for the observation of more subtle phenomena relevant to adhesive interactions. These simulations highlighted the effect of aggregate conformation on adhesive binding potential. Conformations with a more intermediate aspect ratio and/or monomer protrusions along multiple axes, like the triangular triplet, showed increased binding potential relative to doublets or 4-mer rodlike particles. The MAD simulation is particularly well suited for studying these behaviors. Systematically evaluating subpopulations of CTM, like comparing collisions of triangular vs. rod-like triplet aggregates, is not easily accomplished in physical experiments. In the observed experiments, upon forming the first collision, model aggregates pole vaulted to a height greater than their initial position, and they did not form collisions again. Though this behavior is not likely observable *in vitro* or *in vivo*, this behavior does highlight the importance of efficient binding kinetics to promote adhesion under flow. Here, contact time was driven by fluid shear rate, but in physiological environments, CTCs and CTM are brought into contact with the endothelium through collisions with other blood cells, in a process known as margination. Total CTC and CTM contact time is driven by the rate and speed of those collisions, which are dependent on several environmental factors, such as patient hematocrit level [36]. Results from simulation experiments on the effect of contact time on adhesion potential could be used to aid our understanding of the effects of certain patient conditions on observed differences in metastatic behavior. The most computationally expensive component of the MAD simulation is the Monte Carlo simulation of receptor-ligand bonding, so the use of only the hydrodynamic component of the simulation results in faster computing times. The hydrodynamic component of the MAD simulation can thus be initially utilized as a method of evaluating a wide range of parameters to find flow conditions and particle geometries that further support or inhibit binding interactions, and the tandem use of the hydrodynamic and adhesive components of the MAD simulation would allow for more focused analysis of these conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic diagram of Multiparticle Adhesive Dynamics simulation. Linear shear flow brings the aggregate within a reactive distance of the plane wall, at which point a hydrodynamic collision occurs. The major axis, minor axis, and centroid height of the aggregate are highlighted, and the particle surface roughness (ϵ_p) and plane surface roughness (ϵ_w) layers are shown. Reactive distance is defined here as any distance between the roughness layers ≥ 20 nm, the equilibrium length of a sLe^x/E-selectin bond.

Fig. 2.

Generation of model circulating tumor microemboli. Model aggregates used in the MAD simulation were created by modifying a 96-element QUAD9 sphere mesh. The four central elements of a given side of the sphere were removed (A), and that sphere was then duplicated. The duplicated sphere was then reoriented so that the open faces of each sphere were aligned (B). The nodes along the open face of each sphere were then merged so that the resulting aggregate contained one continuous set of nodes (C). This process could be repeated for any face of any sphere, in order to create the desired aggregates. The nodes could also be manually adjusted in order to alter the geometry of the aggregate.

Fig. 3.

QUAD9 element models of Colo205 aggregates. QUAD9 element meshes were generated for doublet, triplet, and 4-mer aggregates using a MATLAB algorithm developed to create particle geometries using a 96 element sphere mesh as a template. Aggregates were created as clusters of spheres of equal size, and then the nodal coordinates were adjusted so that the model particles (A–C) matched the appearance of cultured Colo205 aggregates (D–F) imaged using an inverted microscope.

Fig. 4.

QUAD9 element sphere aggregates. Model aggregates of equal sized spheres were created for simulations of CTM behavior in Stokes flow. Aggregates consisted of clusters of 2, 3, or 4 cells in common geometrical configurations. Particles in (A–F) contained 184, 272, 272, 360, 352, and 360 QUAD9 elements, respectively.

Fig. 5.

Optical and scanning electron microscopy images of Colo205 cells. Representative results of optical and scanning electron microscope (SEM) imaging used to quantify Colo205 morphology. For optical images (A), the line tool in ImageJ was used to measure the lengths of the major and minor axes of individual Colo205 cell in order to determine mean cell diameter. For SEM images (B), the oval selection tool in ImageJ was used to outline the edge of the imaged cells, and the multi-point selection tool was then used to mark the base and peak of surface roughness elements. The distance between the paired points was measured to determine the mean height of the roughness features extending from the surface of the cell.

Fig. 6.

Rotation rate of model aggregates matches analytical solutions. The normalized angular velocity vs. orientation angle of rod-like aggregates placed far from a bounding wall was found to be in excellent agreement with solutions given by Eqs. (2.2) and (2.5). The maximum rotation occurred when the aggregates were aligned perpendicular to flow ($\theta = 0, \pi, 2\pi, 3\pi, \dots$), and the minimum occurred when aggregates were aligned parallel to flow ($\theta = \frac{\pi}{2}, \frac{3\pi}{2}, \frac{5\pi}{2}, \frac{7\pi}{2}, \dots$).

Fig. 7.

Stokes drag force of aggregates matches analytical solutions. The Stokes drag force of aggregates placed far from a bounding wall was found to be in excellent agreement with analytical solutions given by Eq. (16). The relative error for all aggregates was less than 1%.

Fig. 8.

Model circulating tumor microemboli flow behavior is unaffected by shear rate. The trajectory of height of the lowest node point of tetrahedron 4-mer particles, normalized by volume-equivalent sphere radius, was plotted as a function of dimensionless time. Model CTM move in a repeating trajectory particular to its conformation and initial orientation, with the low point decreasing and increasing as aggregate monomers move towards and away from the surface. This pattern was independent of the fluid shear rate; only minor differences were observed in the local maxima and minima of low point heights. A collision event, outlined by the dashed box, corresponds with a blunting of the low point trajectory. During a collision the particle makes contact with the surface, and while the particle still translates and rotates, the low point bottoms out at a minimum, until the particle moves away from the surface and resumes an oscillating pattern. For nondimensionalization, the low point was normalized by the volume-equivalent sphere radius and time was normalized by shear rate.

Fig. 9.

Hydrodynamic simulations are stable over an extended period of time. Dimensionless centroid height trajectory of a model CTM doublet as a function of dimensionless time. Shear rate = 1000 s^{-1} . The model particle oscillated about its initial centroid height of $1 R_e$ with a steady pattern for an extended period of time. For nondimensionalization, the centroid height was normalized by the volume-equivalent sphere radius and time was normalized with shear rate.

Fig. 10.

Model circulating tumor microemboli undergo collisions then exhibit pole vaulting behavior. (A) Time-lapse trajectory of a triangular triplet with an initial centroid height $0.25 R_e$ from the plane surface in flow, shear rate = 2000 s^{-1} . Fluid flow induced particle translation and rotation, which brought aggregate monomers within a reactive distance of the plane surface. (B) Contact with the surface corresponded with the dimensionless centroid height reaching a minimum. Upon forming contact with the surface, the particle pushed off of the surface, and the trajectory stabilized at a centroid height higher than its initial position. This particle motion is referred to as a “pole vault”. (C) The dimensionless velocity was proportional to centroid height, and decreased during the collision and then increased as the particle pole vaulted. For nondimensionalization, the height was normalized by the volume-equivalent sphere radius, time was normalized by shear rate, and particle velocity was normalized by the fluid flow velocity at the initial centroid height.

Fig. 11.

Collision maps of model circulating tumor microemboli particles. Collision maps comprising the cumulative collision events for doublet (A–B), rodlike triplet (C–D), and square 4-mer (E–F) aggregate geometries for simulations with shear rates = 500, 1000, and 2000 s^{-1} . Increased color intensity was proportional to increased total contact time. (A), (C), and (F) display contact events that occurred on the leading edge of the aggregate, and (B) and (D) display contact events that occur on the trailing edge of the aggregate. The view in (F) highlights the fact that collisions did not occur on any other edge of the particle.

Fig. 12.

Collision efficiency is a function of shear rate and particle geometry. Comparison of total contact duration (A), maximum contact area (B), and time integral of contact area (TICA) as a function of shear rate. Increased shear rate decreases the duration of a collision, although the surface area within reactive distance of a given collision (B) remains constant. TICA (C) represents a relative measure of adhesion potential, with a higher number correlating to an increased potential to undergo a reactive event. It combines the contribution of contact area and surface area into one metric.

Fig. 13.

Collision occurrence for randomly oriented particles. Comparison of collision occurrence for model particles, with 3 random particle orientations per initial centroid height. For initial heights marked with an “X,” at least one particle in the group experienced a collision with the surface. For initial heights marked with a circle, no collisions were observed during the entirety of the simulation.

Fig. 14.

Model circulating tumor microemboli adopt stable trajectories after forming collisions.

Trajectories of randomly oriented rod-like triplet with initial centroid height of 0.25, 0.5, 1 R_e freely rotating and translating in fluid with shear rate = 1000 s^{-1} . The particles with the two lowest initial heights formed collisions, which corresponded to the centroid trajectories bottoming out at a minimum. Despite the trajectories reaching different minima, upon pole vaulting their trajectories stabilized at similar heights. For all particles with centroid heights above $0.5 R_e$, no collisions were observed, and centroid heights oscillated with repeating patterns at consistent heights.

Fig. 15.

Comparison of collision metrics for randomly oriented particles. For model CTM conformations where at least one arbitrarily oriented particle formed collisions with the plane surface, total contact time (A), maximum contact area (B), and mean time integral of contact area (TICA) (C) collected for each initial orientation were averaged. Average values were plotted as mean $\pm$ standard deviation. Collisions were observed only for triplet and 4-mer aggregates at initial centroid height of 0.25 or 0.5 R_e , with fluid shear rate = 1000 s^{-1} for all initial heights. The conformations that formed collisions had similar total contact times, max contact area, and adhesion probability, as indicated by similar TICA values. Averaging the collision metrics of arbitrarily oriented aggregates minimized the differences in adhesion probability seen when the triplet-triangle, triplet-rod, 4-mer-square, and 4-mer-

tetrahedron were initially oriented horizontally. The doublet and rod-like 4-mer, in contrast, did not form collisions with any of the initial orientations simulated.

Table 1

Stokes drag force equation shape factor values.

Aggregate shape	Shape factor
OO	$K = 1.12$
OOO	$K = 1.27$
OOO	$K = 1.16$
OOOO	$K = 1.32$
OOOO	$K = 1.17$

Table 2

Relevant physical characteristics of model circulating tumor microemboli. Volume-equivalent sphere radius, aspect ratio, and ellipsoid-equivalent axis ratio are common metrics used to characterize nonspherical particles. Aspect ratio is defined as the ratio between the longest and shortest particle axis. The ellipsoid-equivalent axis ratio is defined as the aspect ratio of an ellipsoid of the same volume. For a chain of spheres, $r_e = 1.14a_r^{0.844}$, where a_r is the true aspect ratio. The ellipsoid equivalent axis ratio was not calculated for the triangular triplet, or 4-mer rod, square, or tetrahedron, as those conformations differed too greatly from an ellipsoid to make a meaningful comparison.

Model particle	Volume-equivalent sphere radius R_e (μm)	Aspect ratio	Ellipsoid-equivalent axis ratio r_e
Doublet	8.762	1.929	1.929
Triplet-triangle	10.028	1.929	
Triplet-rod	10.028	2.859	2.881
4-mer-rod	11.036	3.788	3.673
4-mer-square	11.033	1.929	
4-mer-tetrahedron	11.054	1	

Table 3

Values of input parameters used in Multiparticle Adhesive Dynamics simulation. Key input parameters used in the MAD simulation. Values relating to cellular features were specifically measured for Colo205 cells. Cell radius was determined by measuring the diameters of cultured Colo205 cells imaged with an inverted microscope, assuming cells were spherical. Room temperature was used for MAD simulation experiments. Particle surface roughness was determined by measuring the height of surface roughness features imaged with a scanning electron microscope. Repulsive force parameters were determined through model fitting. The sources of published experimental parameters are cited in the table.

Parameter	Definition	Value	Reference
R	Cell radius	6.959 μm	
F_o	Magnitude of repulsive force	2.4×10^9 pN	
τ	Repulsive force interaction range	$1666.667 \mu\text{m}^{-1}$	
μ	Fluid viscosity	1.0 cP	[26]
ρ_f	Fluid density	$1.0 \times 10^{-6} \mu\text{g}/\mu\text{m}^3$	[26]
ρ_p	Particle density	$1.05 \times 10^{-6} \mu\text{g}/\mu\text{m}^3$	[26]
T	Temperature	298 K	
e_w	Wall surface roughness	350 nm	[11]
e_p	Particle surface roughness	270.6 nm	